

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02905-02 BPS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of β -Amyloid on Ion Transport Across Cell Membranes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Ehrenstein, Ph.D.

Chief, BPS

BPS, NINDS

Others: A. Mbuyi-Kalala, D.Sc.

Visiting Associate

BPS, NINDS

COOPERATING UNITS (if any)

Instrumentation and Computer Section, NINDS (D. Lange)

Laboratory of Neurosciences, NIA (Z. Galdzicki, R. Fukuyama, K.C. Wadhwani, S.I. Rapoport)

LAB/BRANCH

Basic Neurosciences Program, IRP

SECTION

Biophysics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

1.0

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have previously shown that the incubation of PC12 pheochromocytoma cells with β -amyloid for 24 hours causes a significant increase in the choline conductance of the cells. If a similar effect occurs in cholinergic neurons of patients with Alzheimer's disease (AD), the leakage of choline out of the neurons would decrease synthesis and secretion of acetylcholine. This scenario has two important implications. First, the decreased secretion could explain the decreased concentration of acetylcholine found in the brains of AD patients. Second, in view of recent evidence that a decrease in acetylcholine concentration causes an increase in β -amyloid production, there would be positive feedback between decreased acetylcholine concentration and increased β -amyloid concentration. We have developed a mathematical model that includes this positive feedback. According to the model, acetylcholine concentration declines with age in a manner that is very sensitive to the rate of production of β -amyloid. For example, a 10% increase in the rate of β -amyloid production can lower the age of onset of AD, modeled as the age at which the acetylcholine concentration declines to half of its initial value, from 110 years to 60 years. This sensitivity could explain the wide variability of the age of onset of sporadic AD.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 N5 02709-10 BPS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Secretion of Neurotransmitters and Hormones

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Ehrenstein, Ph.D.	Chief, BPS	BPS, NINDS
Others:	A. Mbuyi-Kalala, D.Sc.	Visiting Associate	BPS, NINDS
	M. Jia, M.D.	Visiting Associate	BPS, NINDS

COOPERATING UNITS (if any)

Mayo Clinic, Rochester, MN (L.A. Fitzpatrick)
Naval Medical Research Inst., Bethesda, MD (S.L. Pocotte)

LAB/BRANCH

Basic Neurosciences Program, IRP

SECTION

Biophysics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	1.5	PROFESSIONAL:	1.2	OTHER:	0.3
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously presented evidence that parathyroid cells contain voltage-independent calcium channels and that the function of these channels is to provide a pathway for calcium to enter the intracellular compartment and affect the rate of secretion of parathyroid hormone (PTH). We have now measured PTH secretion in the presence of various channels agonists and antagonists to confirm the presence of the calcium channels. Addition of either of the agonists Bay-K-8644 or (+)202-791 resulted in increased calcium uptake and reduced PTH secretion, whereas the antagonist (-)202-791 caused reduced calcium uptake and increased PTH secretion. Also depolarization of parathyroid cells by applying 50 mM potassium to the extracellular solution increased PTH secretion, and this increased secretion was not altered by either the agonist (+)202-791 or the antagonist (-)202-791. This suggests that the effect of depolarization was to reduce the intracellular calcium concentration enough to saturate PTH secretion. Overall, the experiments with the calcium channel agonists and antagonists confirmed the presence of calcium channels in parathyroid cells and their role in affecting PTH secretion.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02218-20 BPS

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sources and Effects of Reactive Oxygen Intermediates in the Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. L. Gilbert, Ph.D.

Research Physiologist

BPS, NINDS

Others: J. Snell, M.S.

Pre-IRTA

BPS, NINDS

COOPERATING UNITS (if any)

Georgetown University, Washington, DC (C. A. Colton, O. Chernyshev)

LAB/BRANCH

Basic Neurosciences Program, IRP

SECTION

Biophysics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.3

PROFESSIONAL:

2.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiments have been performed on microglia and astrocytes cultured from cerebral cortices of mice and hamsters. We have previously demonstrated that activated rat microglia cells produce the reactive oxygen species (ROS), superoxide radical anion, within a few hours after stimulation and nitric oxide, after a period of about 10 hours to several days. Previously, we have also shown that hamster microglia releases little or no nitric oxide. We have now shown that activated mice microglia also produce nitric oxide. We have continued these studies using normal human microglia, obtained from biopsy samples, and have shown that these human microglia produce little or no nitric oxide. We have tested more than 24 chemical activators including β -amyloid (1-40) to determine if any one of them could activate hamster microglia to produce nitric oxide. Neither hamster nor human microglia produce NO. Human microglia have only been tested with 6 different chemical activators to determine if any of these would be successful techniques for producing nitric oxide. Thus, we have now shown that hamster microglia are similar to human microglia, and that for animal disease models in which microglia participate, it might be better to use hamsters instead of rats and mice. Since arginase catalyses the breakdown of arginine into urea and ornithine, we tested whether the inhibition of this pathway by (+)-S-2-amino-5-iodoacetamidopentanoic acid (AIAP), an inhibitor of the enzyme, arginase, could possibly produce nitric oxide production in the hamster microglia. Preliminary experiments indicated that this inhibition did produce nitric oxide from stimulated hamster microglia. The B103 cells, derived from neuroblastoma line, are exceptional in their lack of β -amyloid precursor protein. The cells have been grown in the presence of hydrogen peroxide as a oxidative stress. We are currently accessing the damage produced by this oxidative stress. We plan to give the same oxidative stress to these cells in the presence of added β -amyloid precursor protein and determine if this is an antioxidant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02890-03 SMS
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Calcium Channels in Vertebrate Nerve Terminals		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	E.F. Stanley, Ph.D.	Staff Physiologist SMS
Others:	Yuan Liu, Ph.D.	Senior Staff Fellow SMS
	Paul Joseph Church, Ph.D.	IRTA Fellow SMS
	Wolfram A. Gottschalk	Pre-IRTA Fellow SMS
COOPERATING UNITS (if any) P. Haydon, Ph.D. Department of Zoological and Molecular Biology, Univ. Iowa, Ames, IA, H. Chin, Ph.D., Staff Scientist, LNC, NINDS		
LAB/BRANCH 		
SECTION Synaptic Mechanisms Section, BNP, DIR		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892.		
TOTAL STAFF YEARS:	4.8	PROFESSIONAL: 4.8 OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Information is transferred from one neuron to the next at <u>synapses</u> , points of intimate contact, by the release of a <u>chemical neurotransmitter</u> . While it is well established that the entry of <u>calcium</u> through ion channels is a critical step in the release of the transmitter, the mechanism and the modulation of this process remains poorly understood. We have previously demonstrated that the <u>calyx-type presynaptic terminal</u> of the chick ciliary ganglion can be used to record single calcium channel activity at a pre-synaptic nerve terminal release face. We have used this preparation to show that single quanta of transmitter can be released during <u>single calcium channel activity</u> . This result is strong evidence that the calcium channel and the transmitter release mechanism are very closely situated, presumably as part of a multimolecular complex. The calyx was also used to demonstrate a regular spacing of individual calcium channels in the transmitter release face using <u>atomic force microscopy</u> . We have also made the first direct recordings of <u>ligand-gated ion channels</u> , activated by <u>ATP</u> , from a presynaptic nerve terminal. These channels may be important in the feedback modulation of transmitter release.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02151-21 LAS

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Memory Storage in Neural Networks

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.L. Alkon, M.D. Medical Officer BNP, DIR, NINDS

Others: T. Nelson, Chemist; D Dahl, Biologist; R. Etcheberrigaray, Vis. Sci; C Collin, Vis. Sci.; M Segal, Vis. Sci; B. Schreurs, Sr. Staff Fellow; J. Olds, Sr. Staff Fellow; O Zohar, Vis. Fellow; N Hirashima, Vis. Fellow; A Favit, Vis Fellow; N Meiri, Vis Fellow; C Yi, Vis Fellow; J Kim, Vis. Fellow; K.L. Blackwell, Guest Res.; D Lester, Guest Res; G Ascoli, Guest Res; J Payne, Guest Res; C Kim, Spec. Vol; C Hirashima, Spec. Vol; M Oh, Spec. Vol.; A Hutter, Spec. Vol.; V Kowtha, Spec. Vol; C Kerns, Spec. Vol.

COOPERATING NITS(if any)

Marine Biological Laboratory, Woods Hole, MA 02543 (A. Kuzirian, P. Smith); Univ. of Chile (I. Atwater, E. Rojas)

LAB/BRANCH

Laboratory of Adaptive Systems

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

17.85

PROFESSIONAL:

16.85

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our principal objective is to define molecular and biophysical mechanisms of associative learning and memory. Emphasis is placed on learning and memory which can be related to human cognition. Ultimate goals are to arrive at clinically meaningful interventions and to design and construct artificial intelligence which has advanced learning and memory capabilities. With human cognitive function as the principal frame of reference, the research focuses on associative processes (such as Pavlovian conditioning) rather than nonassociative behavioral modifications (such as sensory adaptation, habituation, arousal, and sensitization). The biological basis of learning and memory is of interest at several levels of complexity: behavior, neuronal systems, neuronal architecture and membranes and molecular transformations. To reconstruct the physiology involved (and to model it in artificial contexts) it is necessary to use both "simple system" preparations such as the nudibranch mollusk *Hermisenda crassicornis* as well as "complex system" preparations such as rabbits and rats. The molluscan work has yielded the first unequivocal biological record of an associative memory. This record consists of persistent transformations of specific ionic channels. Because these records have been found within the membranes of identified single neurons, it is now possible to define biochemical pathways regulating such long-term membrane modifications as well as to analyze how this biological memory record is expressed by the integrative functions of an entire neuronal system. The work on the vertebrate brain offers two essential opportunities. First, the generality of mechanisms determined for much less evolved species can be tested. Remarkably, the same ionic channel transformations were shown to record associative memory in the rabbit as were found in *Hermisenda*. Rabbit and now rat neural systems have also provided sufficient quantities of tissue so that conditioning-specific alterations of critical enzymatic (e.g., protein kinase C) pathways which control membrane excitability have recently been demonstrated. Furthermore, identical G protein substrates which regulate similar K⁺ channels, intraaxonal transport, mRNA turnover, and architecture of dendritic trees, undergo memory-specific modification in mollusks and mammals. Such biophysical and molecular parallels in mechanisms of memory storage suggest the possibility of general cellular principles of memory storage significant for human physiology and pathophysiology as well. These identified conserved mechanisms of associative memory are guiding a program to uncover targets of dysfunction in Alzheimer's disease for purposes of diagnosis, therapy, and prevention.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 00969-31

LCNSS

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chronic CNS Disease Studies: Slow, Latent, and Temperate Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Carleton Gajdusek, M.D.	Chief	LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief	LCNSS
	David M. Asher, M.D.	Research Medical Officer	LCNSS
	Paul Brown, M.D.	Medical Director	LCNSS
	Ralph M. Garruto, Ph.D.	Supv. Research Biologist	LCNSS
	Richard Yanagihara, M.D.	Medical Director	LCNSS

COOPERATING UNITS (if any)

Continued

LAB/BRANCH

Laboratory of Central Nervous System Studies

SECTION

INSTITUTE AND LOCATION

NINDS, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

18.9

PROFESSIONAL:

11.25

OTHER:

7.65

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies focus on the causes and pathogenesis of chronic degenerative CNS disorders with emphasis on multiple sclerosis (MS); Parkinson's, Pick's, Huntington's and Alzheimer's diseases; ALS; ALS/PD of Western Pacific; supranuclear palsy; other presenile dementias; spinocerebellar ataxias; epilepsy; chronic encephalitis with focal epilepsy; Viliuisk encephalopathy; muscular dystrophies; chronic schizophrenia; bipolar psychoses, autism; SSPE; PML; dialysis encephalopathy, goiterous cretinism; cysticercosis; and intracranial neoplasms. We have defined the transmissible and nontransmissible dementias as brain amyloidoses caused by posttranslational modification of a specific host precursor protein to amyloid fibril deposits. We now recognize the slow-unconventional-viruses causing kuru-CJD-scrapie as replicating polypeptides formed *de novo* from a normal host precursor protein, specified on chromosome 20 in man and 2 in mice. The molecular elucidation of the spontaneous conformational change to infectivity, basically a crystallographic problem, is now becoming our major target. Molecular genetic analysis of familial CJD already indicates several point mutations which enormously increase (x10) the probability of this spontaneous *de novo* conversion to an infectious polypeptide. Microbiology must now contend with a totally new paradigm for replicating, infectious, pathogenic agents in the transmissible brain amyloidoses. Our studies focus on the elucidation of the molecular configurational events conferring the property of infectivity on a previously normal host precursor using CD spectrophotometry, high-voltage EM, MRI to elucidate the change in conformation which occurs as transmissibility is produced. In normal aging, Alzheimer's disease (AD), and Down's syndrome, a different host precursor protein (specified on chromosome 21 in man, 16 in mice) is a cell-excreted inhibitor of growth factors (protease nexin II). Posttranslational degradation of this normal precursor forms the 42-amino acid amyloid polypeptide which polymerizes to form the deposits of amyloid angiopathy, amyloid plaques and neurofibrillary tangles in aging, AD and Down's. This occurs in all individuals who reach their 90s. Genetic, toxic, and infectious factors may accelerate this aging brain amyloid deposition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01282-31 LCNSS
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Neurobiology of Population Isolates: Study of Child Growth, Development, Behavior and Learning, and Disease Patterns in Isolated and Primitive Groups		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
PI:	D. Carleton Gajdusek, M.D.	Chief LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief LCNSS
	David M. Asher, M.D.	Research Medical Officer LCNSS
	Paul Brown, M.D.	Medical Director LCNSS
	Ralph M. Garruto, Ph.D.	Supv. Research Biologist LCNSS
	Richard Yanagihara, M.D.	Medical Director LCNSS
COOPERATING UNITS <small>(if any)</small> Continued		
LAB/BRANCH Laboratory of Central Nervous System Studies		
SECTION		
INSTITUTE AND LOCATION NINDS, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 13.7	PROFESSIONAL: 9.05	OTHER: 4.65
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>Studies of human biology of vanishing primitive societies focus on neurologic development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Opportunistic investigation of problems phrased by man in isolation is the basis of approach from which most of our studies evolved: <u>kuru-CJD GSS-FFI, HIV (AIDS), HTLV-I slow virus infections of the CNS, aging and Alzheimer's, VE, ALS/PD, mental disease, toxic neuropathies.</u> Techniques of molecular genetics, biochemistry, immunology, virology, and field epidemiologic, clinical linguistic and behavioral studies in cultural isolates and genetic and/or geographically isolated primitive bands yield more easily interpretable data than in cosmopolitan societies. Data and specimens from expeditions to Micronesia, Melanesia, Polynesia, South America, Asia and Africa were valuable in recent HIV (AIDS), HTLV-I hantavirus, JCV of PML and herpes virus, CMV and EBV studies. Studies on nutrition, reproduction, fertility, age of puberty and aging, genetic distance and pleomorphisms, unusual and odd higher cortical functions in language learning, cognitive styles, computation (calculation without words or numbers) and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we cannot investigate once the natural cultural experiments in primitive human isolates are amalgamated into the cosmopolitan community of man. Foci of high incidence of kuru, ALS/PD, HTLV-I myelopathy, epilepsy, familial parkinsonism, <u>Viliuisk encephalopathy</u>, other CNS degenerations, hysterical disorders, schizophrenia, bipolar psychoses, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis, and other infections in these isolated groups have yielded widely significant discoveries. HFRS caused by <u>hantaviruses</u> in Asia, USSR, Europe and newly recognized hantaviruses in the U.S. are studied. Human evolution and adaptability to high altitude, various climes, variable food supply, mineral deficiencies, toxic exposures and responses to severe diseases or social psychologic stress are studied in appropriate populations. Thus, HTLV-1 and HIV retroviruses as causes of CNS diseases in man were first found and are best studied in isolated or socially segregated groups: high incidence TSP focus in Tuamaco, Colombia; drug-using mothers in Newark, New Jersey; epidemic neuropathy in Cuba. We now have a proto-Melanesian variant of HTLV-I in New Guinea and Solomon Islands, of an archaic origin, not associated with monkeys at least for millenia.</p>		

PRINCIPAL INVESTIGATORS: (continued)

Others:

Irina Vasilyevna Alekseeva, Ph.D.	Guest Researcher
Luck Ju Baek, Ph.D.	Guest Researcher
Richard Benfante, Ph.D.	Special Expert
Larisa Cervenakova, M.D.	Visiting Fellow
Joab Chapman, M.D.	Special Volunteer
LiJu Fan, Ph.D.	Visiting Associate
Masaya Hironishi, M.D.	Visiting Fellow
Gary Hsieh, Ph.D.	Pre-IRTA Fellow
Stuart Isaacson, M.D.	Clinical Associate (SF)
Bruce K. Johnson, Ph.D.	Special Expert
Kimbra Kenney, M.D.	Medical Staff Fellow
Radzislaw M. Kordek, M.D.	Guest Researcher
Lubica Kurillova, Ph.D.	Visiting Fellow
Pawel P. Liberski, M.D., Ph.D.	Guest Researcher
Vivek R. Nerurkar, Ph.D.	Visiting Associate
Paola Pergami, M.D.	Visiting Fellow
Steven Perlaki, M.D.	Special Volunteer
Jiri Safar, M.D.	Visiting Scientist
Raimundo San Martin, Ph.D.	Visiting Fellow
Jin-Wong Song, M.D., Ph.D.	Visiting Fellow
Chettem Venkateshan, Ph.D.	Visiting Scientist
Ikuro Wakayama, M.D.	Visiting Fellow

Collaborating Units:

Andrew AJDUKIEWICZ, Fiji School of Medicine, Suva, Fiji
Obed ALEMAENA, Min. of Health and Med. Services, Central Hospital, Honiara, Solomon Islands
Vasilii Prokopievich ALEKSEEV, VE Service, Ministry of Health, Sakha Republic, Yakutia
Michael ALPERS, Institute of Medical Research, Goroka, Papua New Guinea
Brian ANDREWS, LNP, NINDS, NIH, Bethesda, MD
P. George BABU, Christian Medical College Hospital, Vellore, India
Courteney BARTHOLOMEW, University of the West Indies, Trinidad
Ivan BASTIAN, Menzies School of Health Research, Darwin, Australia
Roger BAWDON, Department of Obstetrics and Gynecology, Univ. of Texas Med. Center, Dallas, TX
William BELLINI, Center for Disease Control, Atlanta, GA
Abraham BLANK, Universidad del Valle, Cali, Colombia
Luis CARTIER-ROVIROSA, Universidad de Chile, Santiago, Chile
Kwang-Ming CHEN, Guam Memorial Hospital, Agana, Guam
Susan CHENG, EM Facility, NINDS, Bethesda, MD
Chen-Ting CHIN, Beijing University Medical School, Beijing, PRC
S.M. CHOU, Case Western Reserve University, Cleveland, OH
W. CLARK, U.S. Dept. of Agriculture, Mission Field, Mission, TX
David CORBIN, Queen Elizabeth Hospital, Bridgetown, Barbados
Olivia CRUZ, Guam Memorial Hospital, Agana, Guam
Mark DUNCAN, University of New South Wales, Kensington, Australia
Boris Afanasievich EGOROV, Minister of Health, Sakha Republic, Yakutia
Steven FEINSTONE, FDA, CBER, DVP, Bethesda, MD

Judith FRADKIN, NIDDKD, DDEM, Bethesda, MD
 Genoveffa FRANCHINI, LTCB, NCI, NIH, Bethesda, MD
 Blas FRANGIONE, New York University, New York, NY
 Teryl FREI, Georgia State University, Atlanta, GA
 Ryo FUKATSU, Sapporo Medical College, Sapporo, Japan
 Ana GLIGIC, Institute of Immunobiology and Virology, Belgrad, Yugoslavia
 Dmitry GOLDGABER, State University of New York, Stonybrook, NY
 Allen GOLDSTEIN, George Washington University, Washington, DC
 Jaap GOUDSMIT, University of Amsterdam, Amsterdam, The Netherlands
 Matt HALTIA, University of Helsinki, Inst. of Pathology, Helsinki, Finland
 Hiroo HOSHINO, Gunma University School of Medicine, Maebashi, Japan
 J. HOURIGAN, U.S. Dept. of Agriculture, Mission Field Sta., Mission, TX
 Chin-Ming HSIANG, Hubei Medical College, Hubei, PRC
 Julianne IMPERATO-McGINLEY, Cornell University Med. College, New York, NY
 Carol L. JENKINS, Institute of Medical Research, Goroka, Papua New Guinea
 Frederick JENSEN, Immune Response Corporation, La Jolla, California
 T. Jacob JOHN, Christian Medical College Hospital, Vellore, India
 P.R. JOHNSON, Georgetown University, Washington, DC
 Yong KANG, University of Ottawa, Ottawa, Canada
 Manuel KOURI, Pedro Kouri Institute of Tropical Medicine, Havana, Cuba
 Renu B. LAL, Centers for Disease Control, Atlanta, GA
 Ho Wang LEE, Institute for Viral Diseases, Seoul, Korea
 Pawel P. LIBERSKI, Medical Academy Lodz, Lodz, Poland
 Manuel LIMONTA, Institute of Tropical Medicine, Havana, Cuba
 Eugene O. MAJOR, LVMP, BNP, NINDS, NIH, Bethesda, MD
 N. MANTOR, St. Thomas Hospital, U.S. Virgin Islands
 Pedro MAS, Pedro Kouri Institute of Tropical Medicine, Havana, Cuba
 F. MILLER, Arthritis and Rheumatism Branch, NIAMS, NIH, Bethesda, MD
 R.C. MILLER, National Naval Medical Center, Bethesda, MD
 Hiroko MINAGAWA, Kyushu University School of Medicine, Fukuoka, Japan
 Isao MIYOSHI, Kochi Medical School, Kochi, Japan
 Owen St. Cloud MORGAN, University of West Indies, Kingston, Jamaica
 Robin MUKHOPADHYAYA, LTCB, NCI, NIH, Bethesda, MD
 Kurt NOLTE, University of New Mexico, Albuquerque, NM
 Ralph PETERSON, Cornell University Medical College, New York, NY
 P. PLOTZ, Arthritis and Rheumatism Branch, NIAMS, NIH, Bethesda, MD
 Bernard POIESZ, Health Sciences Center, State Univ. of New York, Syracuse, NY
 Stanley RAPOPORT, LN, NIA, NIH, Bethesda, MD
 Patrick REDIG, Raptor Center, St. Paul, MN
 Peter P. ROLLER, DCT, NCI, Bethesda, MD
 George RUBEN, Dept. of Biological Sciences, Dartmouth College, Hanover, NH
 Naruya SAITOU, National Institute of Genetics, Mishima, Japan
 Andres SALAZAR, Walter Reed Army Medical Center, Washington, DC
 Jonas SALK, Salk Institute, La Jolla, CA
 Raymond C. SANDERS, Institute of Medical Research, Goroka, Papua New Guinea
 Duane SCHLITTER, Carnegie Museum of Natural History, Pittsburgh, PA
 Julio SOTELO, National Institute of Neurology and Neurosurgery, Mexico
 City, Mexico
 Peggy SWOVELAND, University of Maryland, Baltimore, MD
 Carol SWYT, BEIB, NIH, Bethesda, MD
 Robert TRAUB, Smithsonian Institution, Washington, DC
 Theodore TSAI, Center for Disease Control, Ft. Collins, CO
 Gordon A.H. WELLS, Ministry of Agriculture, Fisheries and Food, Surrey, U.K.

Charles WEITZ, Temple University, Philadelphia, PA
Afanasii Ivanovich VLADIMIRTSEV, VE Service, Ministry of Health, Sakha
Republic, Yakutia
Vsevolod Afanasiech VLADIMIRTSEV, VE Service, Ministry of Health, Sakha
Republic, Yakutia
Yoshiro YASE, Division of Neurological Diseases, Wakayama Med. College,
Wakayama, Japan
Masayuki YASUI, Division of Neurological Diseases, Wakayama Med. College,
Wakayama, Japan
Takashi YOSHIKI, Hokkaido University, School of Medicine, Sapporo, Japan
Vladimir ZANINOVIC, Universidad del Valle, Cali, Colombia

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02917-01 LDN
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> The Role of a Mesodermal Homeodomain Protein During Vertebra Development		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
P.I.	Heinz Arnheiter, M.D.	Visiting Scientist
Others:	Ellen Meier, Ph.D.	Senior Staff Fellow
	Susan Skuntz, B.S.	Biologist
	Atsuo Nakayama, M.D., Ph.D.	FIC Visiting Fellow
		LDN, NINDS
		LDN, NINDS
		LDN, NINDS
		LDN, NINDS
COOPERATING UNITS <small>(if any)</small> Nancy Jenkins, Ph.D., Neal Copeland, Ph.D.; Rudi Balling, Ph.D.; Elizabeth Hustert, Ph.D.; Max Planck, Institute of Immunobiology, Freiburg i.Br., Germany		
LAB/BRANCH Laboratory of Developmental Neurogenetics		
SECTION Mammalian Developmental Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	1.65	PROFESSIONAL: 0.5
		OTHER: 1.15
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>The structures that encase the central nervous system - the vertebra and the skull - develop from somitic mesoderm and from the neural crest. The molecular mechanisms that underlie this developmental process have not been studied widely. We have obtained a <u>murine transgenic insertional mutation</u> with a recessive phenotype characterized by hemivertebrae, vertebral fusions, and fusions between skull and atlas. We now have determined that the insertion occurred in the first intron of the mesodermal homeodomain gene <i>mox1</i>. This gene is expressed in mesoderm as early as the primitive streak stage and later in presomitic mesoderm, differentiating somites, and mesenchyme of the heart cushion, truncus arteriosus and craniofacial neural crest. The insertion was accompanied by a deletion extending beyond the 3' end of the gene. However, genomic P1 clones that contain the entire <i>mox1</i> gene plus additional 3' sequences have allowed us to span the deletion. Since there is no evidence for additional gross rearrangements or deletions in the chromosomal area surrounding the insertion, it is likely that the phenotype is due solely to the disruption of the <i>mox1</i> gene. The phenotype is consistent with a role of <i>mox1</i> in the formation of preskeletal condensations and indicates that related homeodomain proteins such as <i>mox2</i> with partly overlapping expression patterns are not able to compensate for the loss of <i>mox1</i>. Future studies will show whether the role of <i>mox1</i> is primarily in regulating growth rates of mesenchymal cells, thus specifying the time points and locations of condensations and the formation of ossification centers, or has additional function in specifying the identity of structures of the axial skeleton. The results will have impact on understanding skeletal and skull malformations in mice and humans as well.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02918-01 LDN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics of Complex Neurologic Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. : Lynn D. Hudson, Ph.D. Section Chief LDN, NINDS

Others: Uwe Pott, Ph.D. Visiting Fellow LDN, NINDS
Mirjana Tosic, Ph.D. Special Volunteer LDN, NINDS

COOPERATING UNITS (if any)

Heinz Arnheiter, LDN, NINDS; Jeffrey Barker, LNP, NINDS; W. Theodore, ERB, NINDS.

LAB/BRANCH

Laboratory of Developmental Neurogenetics

SECTION

Section of Basic Neurogenetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

0.95

OTHER:

0.35

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of this section is on the discovery and functional analysis of genes involved in the development and operation of the nervous system. Our broad goal is to define how a network of genes acts to bring about a cellular process. Current research focuses on unraveling the genetics of a complex trait, namely epilepsy. As an entry point for defining genetic interactions, we are specifically mutagenizing in mice the gene for GAD, the biosynthetic enzyme of the neurotransmitter GABA. A construct containing an altered GAD gene has been electroporated into embryonic stem cells, and antibiotic resistant clones have been isolated and screened for the presence of an inactivated GAD gene. These experiments should help determine the role of GAD in neuronal communication, as well as enable us to map additional genes that might act in concert with GAD, and determine whether GAD is involved in disorders characterized by disturbed interneuronal signaling.

The transgenic knock-out studies in mice are being complemented by genetic approaches to directly define genes involved with epilepsy in man. One large family with an autosomal dominant pattern of inheritance of epilepsy has been evaluated at the clinical center, under the direction of Dr. W. Theodore. Samples are currently being collected for linkage analysis. When a high degree of linkage is apparent, the region of interest will be subjected to a series of physical and functional tests in order to finally close in on and sequence the affected gene. Positional cloning will also be carried out on additional families with idiopathic epilepsy to identify other genes of the complex network that, when malfunctioning, yields an epileptic phenotype. Knowledge of which gene(s) are affected in various inherited epilepsies, in conjunction with the identification of genes that affect an epileptic phenotype in transgenic mice, will provide insight as to the molecular basis for interneuronal signaling.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02790-07 LDN*

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Insertional Mutations in Transgenic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Heinz Arnheiter, M.D.	Visiting Scientist	LDN, NINDS
Others:	Colin A. Hodgkinson, Ph.D.	Visiting Fellow	LDN, NINDS
	Atsuo Nakayama, M.D., Ph.D.	Visiting Fellow	LDN, NINDS
	Karin Opdecamp, Ph.D.	Visiting Fellow	LDN, NINDS
	Peter Paras	Biologist	LDN, NINDS

COOPERATING UNITS (if any)

Nancy Jenkins, Ph.D.; Neal Copeland, Ph.D.; Eirikur Steingrimsson, Ph.D.; ABL Basic Research Program, NCI-FCRDC, Frederick.

LAB/BRANCH

Laboratory of Developmental Neurogenetics

SECTION

Mammalian Developmental Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	4.75	PROFESSIONAL:	3.6	OTHER:	1.15
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Many congenital abnormalities of sensory organs such as eye and ear-malformations are the result of derailed development due to malfunctioning genes. We have recently discovered a basic-helix-loop-helix zipper transcription factor gene, called *microphthalmia (mi)*, whose mutations in mice are associated with smaller-than-normal eyes, inner ear deafness, and loss of coat pigmentation. We have also isolated the human homolog of this mouse gene, and it turns out that a particular form of human syndromic hearing impairment called Waardenburg syndrome IIa is in fact due to mutations in this gene. In mice, the common denominator of the abnormalities is the aberrant development or plain absence of pigment cells in eye, inner ear and skin, and the same may be true in humans. We have now determined the developmental expression profile of *mi* in wild type and mutant mouse embryos as well as their cultured melanocytes. In wild type mouse embryos, expression starts in cells in the outer layer of the developing optic cup and soon thereafter in a very small number of cells derived from the neural crest. A few hours after expressing *mi*, the majority of these cells start to express *Trp2*, a melanoblast marker, and thus they seem to be committed to the melanocyte lineage. Since in some locations *mi* expression is found in cells located still in the dorsal wall of the neural tube, commitment may start prior to emigration. In embryos homozygous for certain mutant alleles, neural crest-derived *mi*-expressing cells are hardly detectable, and staining for other melanoblast markers such as *Trp2* remains negative. However, the retinal pigment layer cells stay in place and continue to express *Trp2* but not *Trp1* and tyrosinase, two other melanocyte markers that based on in vitro analyses are direct target genes of *mi*. Since after birth, *mi* is no longer expressed in pigment cells except in those of the hair bulbs, it appears that all abnormalities in eyes and ears may be determined prior to birth, whereas abnormalities in skin pigmentation may continue to develop during life. In a collaborative effort, we have also started to determine the precise molecular defects associated with different mutant alleles in mice and men to analyze the biochemical and biological consequences of these defects in vitro and in tissue culture cells. Interestingly, coexpression of different mutant forms of the Mi protein in compound heterozygotes may lead to milder or more severe abnormalities when compared to the corresponding homozygous mice. Future studies will show whether this observation can be explained solely on the basis of formation of dimers between different mutant forms of the Mi protein or whether it implies involvement of other, related basic helix-loop-helix-zipper transcription factors.

*Formerly LVMP.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02742-09 LDN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Viral Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. :	E. Meier, Ph.D.	Sr. Staff Fellow	LDN, NINDS
Others:	H. Arnheiter, M.D.	Visiting Scientist	LDN, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Developmental Neurogenetics

SECTION

Mammalian Development Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	1.05	PROFESSIONAL:	0.9	OTHER:	0.15
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mx proteins, dynamin, VPS1/SPO15, and MGM1 are members of a newly described family of large GTPases ($M_r = 70,000$ to $100,000$) which are approximately 50% identical in their amino-terminal halves but which have diverse biological activities. The alpha/beta interferon induced Mx proteins confer resistance to specific RNA viruses, most notably influenza viruses. The constitutively expressed dynamin proteins are involved in the constriction of endocytotic vesicles. The yeast VPS1/SPO15 protein is essential for exocytosis and spindle pole body separation during meiosis. The yeast MGM1 protein is required for maintenance of mitochondrial DNA. Of specific interest to us are the biological and cellular functions of the vertebrate members of this family: Mx proteins and dynamin.

With regard to Mx proteins, topics of interest include identification of the intracellular pathway Mx proteins play a role in, biochemical characterization of their GTPase activity, and determination of parameters that affect their antiviral activity and specificity. Current work is aimed at identifying and characterizing cellular proteins that interact with Mx proteins.

With regard to dynamin, our work focuses on the generation of a genetic model for the biological role of the neuron specific dynamin-1 in mice through homologous recombination in embryonic stem (ES) cells. We have already generated animals that carry a mutated dynamin-1 allele, and are currently breeding these animals to homozygosity. If a phenotype is observed in homozygous mice, it may help us to understand how irregularities in the endocytic pathway can precipitate neurologic dysfunction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02528-14 LDN*

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Control of Gene Expression in the Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. : Lynn D. Hudson, Ph.D. Acting Chief LDN, NINDS

Others: Jin Kim, Ph.D. Sr. Staff Fellow LDN, NINDS
Claudia Wiese, Ph.D. Visiting Fellow LDN, NINDS
Mukul Ranjan, Ph.D. IRTA LDN, NINDS
Arthur Warrington, Ph.D. Research Volunteer LDN, NINDS
Jo Ann Berndt, B.S. Microbiologist LDN, NINDS

COOPERATING UNITS (if any)

Heinz Arnheiter, Mammalian Development Section, LDN; H. deF. Webster, LEP, NINDS; R. Armstrong, Dept. Anat. & Cell Biol., USUHS; J. Wrathall, Dept. Anat. & Cell Biology.

LAB/BRANCH

Laboratory of Developmental Neurogenetics

SECTION

Section of Basic Neurogenetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 5.95	PROFESSIONAL: 4.8	OTHER: 1.15
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanisms that dictate the final program of gene expression in a fully differentiated cell can be revealed by starting at either end of the regulatory cascade. To examine the series of controls operating on cells of the oligodendrocyte lineage, we have cloned a number of putative transcription factors that recognize one of the final targets of regulation in myelinating glial cells, proteolipid protein (PLP). One such factor named MyTI (myelin transcription factor I) is a novel member of the zinc finger superfamily. Several observations suggest that MyTI may be instrumental in early stages of oligodendrocyte development and myelin production. MyTI message is highly expressed in oligodendrocyte progenitors and absent in mature oligodendrocytes. MyTI protein appears as speckles within the nucleus of progenitors, suggestive of an association of MyTI with spatially segregated functional domains. MyTI has two clusters of DNA-binding domains, one of which binds to a consensus site represented several times in the PLP promoter and also found in other myelin genes. Experiments are underway to explore the physical interactions of MyTI with DNA through bending assays, the *in vivo* function of MyTI through knockout mice, and the nature of the subnuclear domains in which MyTI is segregated by two approaches, an immunocytochemical colocalization of MyTI with other nuclear proteins found in discrete nuclear domains and an interaction cloning scheme using the yeast two-hybrid system. The MyTI gene resides on human chromosome 2; this mapping data may be useful in evaluating patients with inherited white matter disorders that resemble Pelizaeus-Merzbacher disease but do not carry mutations at the PLP locus, as these patients are candidates for mutations in MyTI or one of the other transcription factors that control myelination. An additional member of the MyTI family, which is also expressed in the developing nervous system, has been isolated and mapped to human chromosome 20. MyTI may represent an emerging class of regulatory proteins with a combination of features that predicts a role in coordinating the expression of a set of genes. Characteristic features shared by such proteins would include a structure of multiple DNA-binding domains which are each stabilized by a divalent cation, initial expression that markedly precedes the target gene, and localization within discrete macromolecular compartments of the nucleus. Identifying genes like MyTI that may be responsible for transforming a precursor cell into a myelinating oligodendrocyte is a prerequisite for designing strategies to stimulate remyelination in inherited or acquired white matter disorder.

*Formerly LVMP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT			PROJECT NUMBER Z01 NS 02789-07 LVMP
PERIOD COVERED October 1, 1994 through September 30, 1995			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurotropism of Human Retroviruses			
P.I.:	M. Dubois-Dalcq, M.D.	Chief, LVMP	LVMP, NINDS
Others:	S. Wilt, Ph.D. J.M. Zhou R. Rusten L. Milward, Ph.D.	IRTA Fellow Vis. Ass. Techn. Biol. Lab Technician FIC Visiting Fellow	LVMP, NINDS LVMP, NINDS LVMP, NINDS LVMP, NINDS
COOPERATING UNITS (if any) M. O'Connor, Univ. of Pa, Philidelphia, PA; D. Griffin and S. Wesselingh, Johns Hopkins Medical School, Baltimore, MD.; K. Holmes and S. Gagneten, USUHS, Bethesda, MD., C. Godfraind, Univ. of Brussels, Bel.			
LAB/BRANCH Laboratory of Viral and Molecular Pathogenesis,			
SECTION Section on Neural and Molecular Biology			
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892			
TOTAL STAFF YEARS:	0	PROFESSIONAL:	0
		OTHER:	0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Projected terminated due to departure of principal investigator.			

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02034-23

LVMP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Oligodendrocyte Lineage of Rodents and Man

P.I. :	M. Dubois-Dalcq, M.D.	Chief, LVMP	LVMP, NINDS
Others:	R. Voskuhl, M.D.	Comm. Officer	LVMP, NINDS
	L. Milward, Ph.D.	IRTA	LVMP, NINDS
	R. Rusten	Biologist	LVMP, NINDS
	P. Paras	Biologist	LVMP, NINDS
	J.M. Zhou	Vis. Ass. Techn.	LVMP, NINDS
	K. Nagasato	Special Volunteer	LVMP, NINDS

COOPERATING UNITS (if any)

Patrick Aubourg, Hospital St. Vincent de Paul, Paris/France; E. Ralston, LNB, NINDS; C. Kufta, NB, NINDS; M. O'Connor, Univ. of Pa, Philidelphia, PA; H. McFarland, NIB, NINDS.

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Neural and Molecular Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	<input checked="" type="checkbox"/>	(b) Human tissues	<input checked="" type="checkbox"/>	(c) Neither
<input type="checkbox"/>	(a1) Minors				
<input type="checkbox"/>	(a2) Interviews				

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated due to departure of principal investigator.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02900-02 LENP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tumor Induction and JC Human Polyomavirus Infection in the Neonatal Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	H.G. Ressetar, Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	G.L. Stoner, Ph.D.	Section Chief	LENP, NINDS
	H.deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study investigates cellular alterations and pathogenesis induced by the human polyomavirus, JC virus (JCV) in experimental animals and human tissues. JCV causes the human CNS demyelinating disease, progressive multifocal leukoencephalopathy (PML) and induces brain tumor formation in experimental animals. Immunohistochemical studies have focused on JCV infection of and/or interacting influence on astrocytes, vascular endothelial cells (EC) and neurons. JCV-infected astrocytes were observed in most PML cases examined, with one non-AIDS PML case exhibiting extensive infection of basal ganglia astrocytes. Comparative evaluation of astrocytic markers revealed an apparent progression from early-onset loss of GFAP expression in some astrocytes, to increased expression of vimentin in astrocytes outside of lesion areas and in early focal clusters. GFAP-positive reactive astrocytes surrounded more advanced lesions with some cells double-labeled for GFAP and vimentin. Lesion area vascular alterations were suggested by increased expression of basement membrane components laminin and collagen IV while EC expression of Factor VIII and ICAM-1 was similar to control brains. Cerebellar internal granular layer changes were present in some cases with apparent loss of granule neurons. This was also noted in the hamster model of JCV brain infection in addition to infection and neoplasia of granule neurons with accompanying astrocytosis.

Recent efforts include the development of a mouse model to examine the influence of the HIV TAT protein on JCV-induced pathogenesis. Studies indicate that JCV is expressed in subependymal and subpial cells 3 to 15 days after intracerebral inoculation into newborn mice and in some kidney cells in intraperitoneally-inoculated animals. JCV expression is currently being examined in JCV-inoculated transgenic mice carrying SV40-TAT gene sequences.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02808-06 LENP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Molecular Studies of Growth Factors during Myelin Breakdown and Regeneration in the CNS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	H. Webster, M.D.	Chief	LENP, NINDS
Others:	D. Yao, M.D.	Visiting Scientist	LENP, NINDS
	X. Liu, M.D.	Visiting Fellow	LENP, NINDS
	L. Hudson, Ph.D.	Chief	LDN, NINDS
	C. Bondy, M.D.	Staff Scientist	DEB, NICHD
	M. Brenner, Ph.D.	Staff Scientist	SB, NINDS
	J. Gehrmann, M.D.	Guest Researcher	LENP, NINDS

COOPERATING UNITS (if any)

Laboratory of Developmental Neurogenetics, DIR, NINDS; Developmental Endocrinology Branch, NICHD; Stroke Branch, NINDS; Dep. Neuromorph., Max Planck Inst. Psych., Martinsried, Germany

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	3.55	PROFESSIONAL:	2.8	OTHER:	0.75
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to study the glial gene expression of growth factors, myelin-related proteins and other glial proteins during nervous system injury and regeneration. Previous studies have shown that hypertrophic astrocytes in lesions of acute experimental autoimmune encephalomyelitis (EAE) synthesize insulin-like growth factor I (IGF-I). In addition, during clinical recovery and demyelination, oligodendrocytes (the cells responsible for CNS myelin synthesis) express the IGF-I receptor. The EAE lesions in which IGF-I expression occurs are characterized by breakdown of the blood-brain barrier, immune-mediated inflammation, severe demyelination and relative preservation of axons. Since they closely resemble those seen during active demyelination in multiple sclerosis, studies this year were designed to test whether IGF-I treatment of Lewis rats with this type of EAE would significantly alter clinical deficits and lesion severity. About 12 days after EAE induction, pairs of rats with the same degree of mild but definite weakness were selected and given intravenous placebo or IGF-I twice daily for 8 days. IGF-I treated rats had less severe clinical deficits and began recovering sooner. Compared to placebo-treated rats, those treated with IGF-I had reduced permeability of the blood-brain barrier and fewer inflammatory demyelinating lesions; these lesions also were smaller. In lesions of IGF-I treated rats, there also were fewer demyelinated axons, higher levels of myelin protein messenger RNAs, and more regenerating myelin sheaths. This evidence suggests that IGF-I may be useful in treating patients with multiple sclerosis and other demyelinating diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02849-04 LENP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Human Polyomavirus Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	G.S. Ault, Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	G.L. Stoner, Ph.D.	Chief, Neurotoxicology Section	LENP, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	<input checked="" type="checkbox"/>	(b) Human tissues	<input type="checkbox"/>	(c) Neither
<input type="checkbox"/>	(a1) Minors				
<input type="checkbox"/>	(a2) Interviews				

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated due to reassignment of principal investigator.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02827-05 LENP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification, Characterization, and Etiologic Role of Human Polyomavirus in Neurological Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	M. Ishaq, Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	G.L. Stoner, Ph.D.	Chief, Neurotoxicology Section	LENP, NINDS

COOPERATING UNITS (if any)

Dept. of Molecular and Cell Biology, Penn State University (R.J. Frisque)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	0	PROFESSIONAL:	0	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated due to departure of principal investigator.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02803-06 LENP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Latency and Pathogenesis of Herpes Simplex Virus in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. W.J. Mitchell, D.V.M., Ph.D. Senior Staff Fellow LENP, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS

0.95

PROFESSIONAL:

0.65

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand aspects of the pathogenesis of herpes simplex virus (HSV) infection in the nervous system including the mechanism by which this neurotropic virus is regulated within neuronal and nonneuronal cells during acute and long term infections. A further objective is to understand the relationship between HSV infection and disease. During FY 95 studies were initiated to examine whether the HSV-1 major immediate early gene (ICP4) is transcriptionally active in latently infected ganglia. Transgenic mice containing the HSV-1 ICP4 promoter sequence fused to the E. coli beta galactosidase coding sequence were analyzed after infection with HSV-1. Nonneuronal cells morphologically identified as Schwann cells were positive for beta-galactosidase in latently infected trigeminal ganglia. Neurons were negative for ICP4 promoter activity in latently infected ganglia. This suggests that chronic infections of nonneuronal cells of ganglia are regulated by a different mechanism than latently infected neurons. The viral gene expression in nonneuronal cells of ganglia may be the underlying cause for chronic inflammatory lesions in HSV-1 infected trigeminal ganglia of mice. It is also possible that this mechanism could function in chronic inflammatory diseases of sensory ganglia of humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02804-06 LENP
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Nervous System Regeneration in a Herpesvirus Model		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I. D.B. Henken, Ph.D. Senior Staff Fellow LENP, NINDS Others J.R. Martin, M.D. Medical Officer LENP, NINDS		
COOPERATING UNITS (if any) M.E. Goldstein, Ph.D., Bristol-Myer Squibb Pharmaceuticals, Meriden , CT; R. Curtis, Ph.D., Regeneron Pharmaceuticals, Tarrytown, NY		
LAB/BRANCH Laboratory of Experimental Neuropathology		
SECTION Cellular Neuropathology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Project terminated due to departure of principal investigator.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02549-14 LENP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Herpesvirus Infections and Nervous System Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	J.R. Martin, M.D.	Medical Officer	LENP, NINDS
Others:	S. Keir, Ph.D.	Visiting Fellow	LENP, NINDS
	W.J. Mitchell, D.V.M., Ph.D.	Sr. Staff Fellow	LENP, NINDS
	D.B. Henken, Ph.D.	Sr. Staff Fellow	LENP, NINDS
	H. deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS (if any)

Anatomic Pathology, Texas Childrens Hosp. (C. Langston, M.D.); Dept. of Pediatrics, Univ. of Alabama at Birmingham (E. Kern, Ph.D.)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated due to the departure of principal investigator.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02550-14 LENP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Immunologic Mechanisms in Virally-Induced CNS Demyelination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Gerald Stoner, Ph.D.	Chief, Neurotoxicology Section L	LENP, NINDS
Others:	H.T. Agostini, M.D.	Guest Researcher	LENP, NINDS
	H.G. Ressetar, Ph.D.	Senior Staff Fellow	LENP, NINDS
	C. Ryschkewitsch, B.S.	Medical Technologist	LENP, NINDS
	Y. Shishido, M.D.	Visiting Scientist	LENP, NINDS
	H. deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS (if any)

Lab. Mol. Oncol., Alton Ochsner Med. Fdn. (O. Prakash); Mol.Biol., Penn State U. (R.J. Frisque); VAMC West LA, CA (E.J. Singer, W.W.Tourtellotte, R.W. Baumhefner); Shirati Hosp., Tanzania (G. Brubaker)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.15

PROFESSIONAL:

2.55

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project concerns mechanisms of CNS demyelination in human diseases and continues to focus on the molecular characterization of the human polyomavirus JC virus (JCV), the etiologic agent of progressive multifocal leukoencephalopathy (PML). PML is an infection of oligodendrocytes in the CNS, causing a demyelinating disease which is fatal in about 5% of AIDS patients, usually within 3-6 months. Work this year has continued to emphasize the detection and characterization of JCV by polymerase chain reaction (PCR) amplification from PML tissues, CSF, and from the urine of normal controls and multiple sclerosis (MS) patients. Notable advances this year have included the following: 1) the characterization of JCV and BKV in African urines obtained from AIDS patients in Shirati, Tanzania, including the identification of East African JCV as a new type of the virus (Type 3). The entire genome of several African strains has been amplified by PCR as six separate fragments, and the genome of this new type will be sequenced; (2) A group of urines from 105 control individuals including 5 African-Americans was characterized for the type of JCV present. These were typed as follows: 29 Type 1 strains (1A or 1B), eight Type 2 strains, no Type 3 strains, seven Type 4 strains, and one Type 5 strain. Two individuals were doubly infected with different types or subtypes of JCV. Two possible new types of JCV were identified in these patients. Type 4 consists of an insert of about 150bp from Type 3 into Type 1 sequence. Type 5, with a single example discovered so far, may be derived from a sequence ancestral to Types 2 and 3 to which it is most closely related. (3) Chronic progressive MS patients showed numbers of JCV positive urines comparable to controls, and excretion of the virus was not enhanced by immunosuppressive treatment with cyclosporine. No examples of Type 2 were identified in a group of 37 patients, but this difference in type distribution compared to controls was not statistically significant. Larger numbers of patients, including those with relapsing/remitting disease, will be studied, and the influence of clinical relapse on virus excretion will be examined. (4) A technical advance developed in our Section has allowed cloning the entire JCV genome (5100 bp) from brain, CSF, and urine. This will greatly facilitate cloning and sequencing of variant JCV strains, including those derived from MS patients.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01995-23 LENP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Molecular Studies of Myelin Formation, Breakdown and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	H.deF. Webster, M.D.	Chief	LENP, NINDS
Others:	Q.-L. Zhang, M.D.	Visiting Fellow	LENP, NINDS
	P-X. Lin	Visiting Associate	LNC, NINDS
	J. Liu, M.D.	Visiting Fellow	LENP, NINDS

COOPERATING UNITS (if any)

Laboratory of Neurochemistry, NINDS

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	<input type="checkbox"/>	(b) Human tissues	<input checked="" type="checkbox"/>	(c) Neither
<input type="checkbox"/>	(a1) Minors				
<input type="checkbox"/>	(a2) Interviews				

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been incorporated into Z01 NS-02808-06 LENP.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02919-01LMB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Light Imaging Facility

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Carolyn L. Smith, Ph.D.

Senior Staff Fellow

LMB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Light Imaging Facility (LIF) is a new NINDS-wide facility created to provide access to state of the art light imaging equipment and expertise in light imaging techniques to all laboratories and branches in the Division of Intramural Research. The LIF has a laser scanning confocal microscope designed for high resolution imaging of structure within thick biological preparations, a video microscope with cameras for high resolution and low light level imaging, computers for image procession and analysis and a high quality digital image printer. The LIF functions in two modes: (1) In a collaborative/consultation mode to train and assist scientists in application of light imaging techniques to ongoing research projects and (2) As a research entity to develop and test new light microscopic approaches for studying nervous system development and function. During the eight months since its inception, the LIF has made major contributions to fifteen collaborative research projects involving thirty-two scientists in eleven laboratories (eight in NINDS and three in other institutes). These projects address a wide range of problems in neurobiology as well as other fields of biomedical research. Independent research in the LIF has focused primarily on the development of methods for visualizing and interfering with the function of specific proteins in living cells. Before the end of FY95, the LIF will acquire equipment for a new technique, invented by D. Jay (Harvard University), that uses a laser beam to selectively inactivate antibodies and proteins attached to them. This technique potentially could provide a more flexible and temporally precise method for "knock-out" of specific proteins than conventional genetic approaches and would be a useful tool for work on a wide range of problems.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02881-03LMB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Biology of the Mammalian Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.D. McKay, Ph.D.	Chief	LMB, NINDS
Other:	J.P. Bengzon, M.D., Ph.D.	Visiting Fellow	R. Josephson
	C.O. Brüstle, M.D., Ph.D.	Special Volunteer	Y. Maeda, M.D., Ph.D.
	D.M. Panchision, Ph.D.	IRTA	M.J. Marvin
	L.M. Delgado-Rivera, Ph.D.	Visiting Fellow	S. Okabe, M.D., Ph.D.
	T.E. Hayes, Ph.D.	Senior Staff Fellow	G. Vaughn
	T.G. Hazel, Ph.D.	IRTA	C. Vicario, Ph.D.
			Pre-IRTA
			Visiting Associate
			IRTA
			Visiting Associate
			Biologist
			Visiting Fellow*

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.18

PROFESSIONAL:

3.77

OTHER:

1.41

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The research program in this group is in the area of developmental neurobiology. The adult mammalian brain is composed of a vast number of different neurons. In embryonic development, neurons are derived from multipotential precursor cells. When these precursors stop dividing they become committed to give specific neuronal types in a very precise pattern. This commitment step, which occurs in the few hours around the last division, controls critically important features of neuron numbers and types found in the adult brain. Our work is focused on the molecular and cellular mechanisms regulating this process.

The key methods we currently employ include: (1) the use of transgenic mice to define DNA-sequences that target gene expression to neuronal precursors; (2) dissociated cell and tissue slice culture analysis of growth factors which regulate the proliferation, survival and differentiation of cells in the embryonic brain; and (3) the use of transplanted neuronal precursors to construct chimeric brains carrying genetically engineered functional neurons.

These techniques are used to analyze the molecular mechanisms controlling the development and function of the mammalian brain. The results are applicable to understanding the genetic basis of childhood tumors and neurodegenerative diseases of the central nervous system. They may also lead to powerful new therapies to reconstruct the damaged structure(s) found in Parkinson's, Alzheimer's and Huntington's diseases.

*Continued:

M. Dugich, Ph.D.	IRTA	T. Müller, Ph.D.	Visiting Fellow
T. Hisatsune, Ph.D.	Special Volunteer	K. Forsberg-Nilsson, Ph.D.	Special Volunteer
K. Johe, Ph.D.	IRTA	J.M. Pickel, Ph.D.	IRTA
U. Maskos, Ph.D.	Special Volunteer	P. Tsoulfas, M.D.	IRTA
A.C. Spiro	Pre-IRTA	M. Molne-Monne, Ph.D.	Visiting Fellow

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02884-03LMB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Cycle Regulation During *Drosophila* Visual System Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Brian Mozer, Ph.D.

IRTA

LMB, NINDS

Others: Karen L. Powers

Pre-IRTA

LMB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.33

PROFESSIONAL:

1.0

OTHER:

0.33

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

During the development of the nervous system, the regulation of mitosis in neuronal precursors must be coordinated with their differentiation. A genetic analysis of cell cycle regulation is being pursued in a model system, the developing eye of *Drosophila melanogaster*. In the eye imaginal disc of the third instar larva, photoreceptor cell precursors differentiate in the wake of the morphogenetic furrow. Developmental decisions affecting cell cycle regulation likely occur among retinal precursor cells immediately anterior to the furrow. The transient expression ahead of the furrow of the *stg* (*stg*) gene, the fly homolog of the yeast cell cycle regulator *cdc25*, and the ubiquitous expression of other general cell cycle control genes (*cdc2*, *cyclins*) suggest that the establishment of the *stg*-domain may be a rate limiting step in eye development.

The dominant small eye mutant, *Drop* (*Dr*) has identified a novel gene required for cell cycle regulation during eye development. Based on genetic interactions with mutations in other known cell cycle components, *Drop* likely functions as an upstream activator of *stg*. Development analysis of both gain of function and loss of function phenotypes of *Dr* mutants suggests that the primary defects in cell cycle regulation occurs in imaginal cells. Homozygous *Dr* null mutant embryos are lethal, but exhibit no gross morphological defects, and have normal patterns of cell proliferation and *stg* RNA expression. In contrast, in the eye imaginal disc of a dominant gain of function *Dr* mutant, the expression of *stg* RNA in progenitor cells anterior to the morphogenetic furrow is blocked. The absence of *stg* expression is associated with the failure of morphogenetic furrow movement and lack of photoreceptor cell differentiation.

DNA lesions associated with gain of function and loss of function *Dr* mutations have been localized to an 80 kb chromosomal walk from the distal 99A region of the third chromosome. Northern blot analysis and cDNA library screening have identified three transcripts within the cloned DNA. Experiments are currently in progress to determine which of the three transcripts corresponds to the *Drop* gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02864-04LMB*

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dopaminergic, Neurotrophic Factors and Reinnervation of the Spinal Cord

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.W. Commissiong, Ph.D.	Visiting Scientist	LMB, NINDS
Others:	J. M. Johnston, Ph.D.	Visiting Fellow	NTU, LMCN, NINDS
	P.Tsoulfas, M.D.	IRTA	LMB, NINDS
	R.D. McKay, Ph.D.	Chief	LMB, NINDS

COOPERATING UNITS (if any)

Laboratory of Cellular and Molecular Biology, LMCN, NINDS

LAB/BRANCH

Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.15

PROFESSIONAL:

1.15

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The fundamental mechanism that underlies the degeneration of dopaminergic neurons, leading to the neurologic syndrome of Parkinson's disease remains unresolved, despite three decades of intense research. We have focused on glial sources of dopaminergic neurotrophic factors (DNTFs). The oligodendrocyte type-2 astrocyte (0-2A) progenitor cell and type-1 astrocytes (T1-As), proliferated from the striatum and ventral mesencephalon of the E16 rat brain respectively, are two major sources of DNTFs. We prepared 15L of conditioned media from T1-As, and partially-purified two glycosylated proteins of molecular mass 15 and 50 Kd, that retained dopaminergic neurotrophic activity. However, we failed, for technical reasons, to obtain useful sequence data. A major effort is currently being devoted by several leading laboratories, including our own, to testing the following members of the transforming growth factor (TGF) family of compounds as possible DNTFs, and determining their cellular sources: glial cell line-derived neurotrophic factor (GDNF), TGF- β 1, TGF- β 2 and TGF- β 3. Using RT-PCR, we have identified mRNA for GDNF and TGF- β 1, -2 and -3 in T1-As and B49 cells (source of GDNF), and mRNA for TGF- β 1 and -3 in 0-2A progenitors. Our bioassay data on the efficacy of these growth factors on the survival of dopaminergic neurons in culture show that GDNF is effective, TGF- β 1 is ineffective, and TGF- β 2 and -3 may be toxic. These results differ markedly from recent reports suggesting the possible use of members of the GF family to treat neurodegenerative diseases. There is a critical need for a standardized bioassay to test putative DNTFs. We have developed such an assay, based on a precise, microdissection technique, and the use of microisland cultures and imaging methods. We have developed a new and more efficient method for proliferating 0-2A progenitor cells to 95% purity, and avoids contamination with microglia. The 5% of contaminating cells are mainly T1-As. This pure 0-2A cell preparation will facilitate our research on the isolation of putative DNTFs. It will also greatly facilitate research on the biology of oligodendrocyte precursors, particularly paradigms related to remyelinating studies in the CNS. In collaboration with the Laboratory of Cellular and Molecular Neurobiology (LMCN), we have identified a minor ganglioside that is recognized by the A2B5 antibody, and which may be uniquely localized to oligodendrocyte precursors. This finding may provide the basis for the production of an antibody specific for 0-2A progenitor cells of cortical and striatal origin, and CG4 cells.

*Formerly in LMCN.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02788-06LMB

PERIOD COVERED

October 1, 1994 through Septmeber 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Neuronal Shape

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Carolyn L. Smith, Ph.D.

Senior Staff Fellow

LMB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH. Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is being terminated for Fiscal Year 1995.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02848-04
LMCN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Disorders of CNS Myelin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Johanna R. Möller, M.D.	Unit Head, Special Expert	DDU, LMCN, NINDS
Others:	Jeffrey W. Stebbins, Ph.D.	IRTA	DDU, LMCN, NINDS
	Yukio Arai, M.D.	Visiting Fellow	DDU, LMCN, NINDS.
	Carl J. Lauter	Chemist	LMCN, NINDS
	Jeffrey A. Hammer	Biologist	LMCN, NINDS

COOPERATING UNITS (if any)

LNC-NINDS Protein/Peptide Sequencing Facility; DMNB and LENP, NINDS; Sch. Vet. Medicine, U. of Wisconsin; Pathology Dept., Michigan State U.; Neurology Dept., Kennedy Inst., Baltimore, MD

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Demyelinating Disorders Unit, Section on Myelin and Brain Development

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.7

PROFESSIONAL:

2.4

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In multiple sclerosis (MS), there is a preferential loss of myelin-associated glycoprotein (MAG) at the edges of the plaques. Much of the MAG remaining in the form of dMAG, a proteolytic cleavage product of a myelin-associated, calcium activated neutral protease (calpain). The MAG loss in MS may be related to calpain. dMAG is also present in some patients with AIDS (see Z01NS02805-06 LMCN). The MAG/dMAG conversion rate in incubated myelin from different species is greatest in human myelin, rapid in other primates, and much slower in mammals such as rodents. This suggests that dMAG formation may be relevant to human demyelinating diseases. The MAG/dMAG conversion rate is very sensitive to the Ca^{2+} levels in myelin incubations. Purified human calpain incubated with purified human MAG degraded the MAG totally. To determine the native proteolysis site for calpain, dMAG from three species was chemically purified. Peptide fragments of the isolated dMAG were generated enzymatically and the peptide containing the dMAG carboxyl terminus was purified by reverse phase HPLC and then sequenced. Biochemical analysis of white matter biopsies from 2 young girls with a progressive leukodystrophy, due to unknown causes, revealed the presence of all the characteristic myelin proteins and lipids at very low levels. Differential display assays were started to elucidate the possible cause of the leukodystrophy. In most hypomyelinating mutant animals, myelin basic protein (MBP) and proteolipid protein (PLP) are decreased more than MAG and 2',3'-cyclic nucleoside 3'-phosphodiesterase (CNP), due to a greater deficiency of compact myelin than of associated oligodendroglial membranes. However a new neurological rat mutant, the TAIEP rat, expresses decreased amounts of MAG compared to other myelin proteins. In the younger mutants MAG has a higher molecular weight than in aged-matched controls, most likely due to an extended presence of the immature large isoform of MAG. Total MAG mRNA levels were the same in affected and control rats. PCR of TAIEP samples showed mRNA for both the immature and mature isoforms of MAG. Also in caprine β -mannosidosis, MAG, CNP and PLP levels were equally decreased, but MBP was relatively spared. The presence of large storage vesicles might interfere with the protein transport of MAG, PLP and CNP, while MBP translation is at the site of insertion into the myelin. The mRNA levels for MAG, PLP and MBP were decreased equally to about 50% supporting this hypothesis.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02805-06
LMCN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Immunological Aspects of Myelin Abnormalities in Neuro-AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Johanna R. Möller, M.D.	Unit Head, Special Expert	DDU, LMCN, NINDS
Others:	Jeffrey W. Stebbins, Ph.D.	IRTA	DDU, LMCN, NINDS
	Carl J. Lauter	Chemist	LMCN, NINDS
	Jeffrey A. Hammer	Biologist	LMCN, NINDS
	Richard H. Quarles, Ph.D.	Laboratory Chief	LMCN, NINDS

COOPERATING UNITS (if any)

Medical Neurology Branch, NINDS; Dept. of Neurology, Johns Hopkins University, Baltimore, MD;
Cleveland Clinic, Cleveland, Ohio

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Demyelinating Disorders Unit, Section on Myelin and Brain Development

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.75

PROFESSIONAL:

0.5

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project studies biochemical and immunological aspects of myelin disorders in neuro-AIDS. Myelin-associated glycoprotein (MAG) and CD4 both belong to the immunoglobulin gene superfamily. Its members generally serve in recognition processes, and human CD4 is also the receptor for gp120 of HIV. There is some homology between the binding site of CD4 for gp120 and MAG. Soluble dMAG (a proteolytic cleavage product of MAG containing all five extracellular domains) was chemically purified with the intent to grow native dMAG crystals and to determine their structure by X-ray diffraction. Postmortem subcortical white matter samples from AIDS patients with and without dementia were analyzed for quantitative and qualitative alterations of myelin proteins, including MAG, myelin basic protein, proteolipid protein and 2', 3'-cyclic nucleotide 3'-phosphodiesterase. Diffuse myelin pallor (DMP) was detected histologically (indicated by a decreased staining with luxol fast blue) in about one-half of demented patients and one-fourth of the nondemented patients. The biochemical results were correlated with histological and immunocytochemical observations made by our collaborators at Johns Hopkins University on adjacent tissue sections. However, electron microscopic, immunocytochemical and biochemical analyses of white matter indicated little or no loss of myelin proteins in areas of prominent DMP. The same was found when demented and nondemented AIDS patients samples were compared to controls. Astrocytic hypertrophy was found in some of the AIDS patients both histologically and by increased levels of glial fibrillary acidic protein detected biochemically. Significant accumulation of serum proteins was detected immunocytochemically in white matter of many of the AIDS cases, especially the demented ones. This was supported biochemically by the presence of variable levels of haptoglobin on western blots of AIDS samples but not of control samples. Overall, the results provide little evidence for significant demyelination or myelin pathology in subcortical white matter of AIDS brain, but suggest that blood brain barrier perturbation may contribute to CNS pathology in AIDS and AIDS dementia. Also, substantial conversion of MAG to dMAG was observed in some AIDS samples, as had previously been found in many samples from multiple sclerosis brains (Z01NS02848-04LMCN). The relationship of blood brain barrier breakdown to the proteolytic MAG/dMAG conversion observed in multiple sclerosis and the AIDS brain is under investigation.



NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS 02784-07
LMCN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of Stimulatory Guanine Nucleotide Binding Protein Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.V. Rebois, Ph.D.	Unit Head	LMCN, NINDS
Others:	N.S. Basi, Ph.D.	IRTA Fellow	LMCN, NINDS
	D. Warner, Ph.D.,	IRTA Fellow	LMCN, NINDS
	M. Nishimura, M.D., Ph.D.	Visiting Fellow	LMCN, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Membrane Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892, ,

TOTAL STAFF YEARS:	3.9	PROFESSIONAL:	3.75	OTHER:	0.15
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The stimulatory (G_s) and inhibitory (G_i) G proteins regulate the activity of adenylyl cyclase (AC). Activation of these heterotrimeric ($\alpha\beta\gamma$) G proteins occurs when an agonist-receptor complex promotes the exchange of GDP for GTP in the nucleotide binding site of the α -subunit ($G\alpha$). Under some circumstances, $G\alpha$ dissociates from the $\beta\gamma$ -subunit complex ($G\beta\gamma$). The prevailing hypothesis proposes that subunit dissociation necessarily accompanies G protein activation, and subunit reassociation accelerates G protein deactivation by stimulating the intrinsic GTPase activity of $G\alpha$. Thus, the interplay between $G_s\alpha$, $G_i\alpha$ and $G\beta\gamma$ is critical for controlling the activity of AC. However, subunit dissociation may not accompany G_s activation. G_s was prepared from bovine brain and its activity determined by reconstituting AC in $G_s\alpha$ -deficient *cyc* membranes. G_s subunit dissociation was assayed by immunoprecipitating $G_s\alpha$, and determining the amount of $G\beta$ that was coprecipitated, or by the ability of G_s to serve as a substrate for cholera toxin (CT). CT ADP-ribosylates heterotrimeric G_s but not the free $G_s\alpha$ subunit. We found that in solution with 2 mM Mg^{2+} , both GTP γ S and AlF_4^- activated G_s without causing subunit dissociation. In solution, Mg^{2+} (2-120 mM) caused a dose-dependent dissociation of G_s subunits that was inhibited to the same extent by GDP and GTP. 120 mM Mg^{2+} caused nearly complete G_s subunit dissociation, and inactivated G_s unless guanine nucleotides were present. G_s was incubated in solution with 120 mM Mg^{2+} for 0-2 h in the absence of guanine nucleotides, then GTP γ S was added to stop further inactivation, and to activate any G_s that was not denatured. G_s activated in this way could not be ADP-ribosylated by CT in solution indicating that complete subunit dissociation had occurred ($G_s\alpha$ - GTP γ S plus $G\beta\gamma$). When $G_s\alpha$ -GTP γ S was incorporated into *cyc*, it became a substrate for CT and also stimulated AC. These two phenomenon were closely correlated and inversely related to the length of exposure to 120 mM Mg^{2+} in the absence of GTP γ S. *In vitro* transcription and translation was used to prepare active $G_s\alpha$ ($G_s\alpha_{52}$) and type IV adenylyl cyclase (ACIV) from recombinant DNA. In solution, G_s from bovine brain could activate ACIV, but activation by $G_s\alpha_{52}$ was largely dependent upon $G\beta\gamma$. $G\beta\gamma$ alone had no effect on the activity of ACIV. When *cyc* were depleted of $G\beta\gamma$ by a detergent/salt wash, and reconstituted with $G_s\alpha_{52}$, it was a poor activator of AC and a poor substrate for CT. If $G\beta\gamma$ was included during reconstitution, it caused a concentration-dependent increase in both AC activity and ADP-ribosylation of $G_s\alpha_{52}$, and these two phenomenon were closely correlated. These data suggest that the activated G_s heterotrimer, and not the free $G_s\alpha$ subunit mediates stimulation of AC both in membranes and in solution.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS02786-07LMCN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibodies to Glycoconjugates in Neurological Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	R. H. Quarles, Ph.D.	Section Chief	LMCN, BNP, NINDS
Others:	R. Farrer, Ph.D.	Sr. Staff Fellow	LMCN, BNP, NINDS
	C. Lauter	Chemist	LMCN, BNP, NINDS
	J. Hammer	Biologist	LMCN, BNP, NINDS
	M. Dalakas, M.D.	Section Chief	MNB, CNP, NINDS

COOPERATING UNITS (if any)

Neuromuscular Diseases Section, MNB, CNB, NINDS; Dept. Pathology, State Univ. New York, Syracuse, NY

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP

SECTION

Myelin and Brain Development Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

1.0

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monoclonal anti-myelin associated glycoprotein (MAG) antibodies in patients with mixed sensory-motor polyneuropathies occurring in association with IgM gammopathy (paraproteinemia) are all directed toward carbohydrate epitopes in MAG and cross react with other glycoproteins of PNS myelin including PO and PMP-22 as well as with the glycosphingolipid, sulfate-3-glucuronyl paragloboside (SGPG). In order to gain insights about the pathogenic mechanisms involved, we are biochemically analyzing an animal model of this disease involving passive transfer to chickens by infusion of human anti-MAG antibodies and which exhibits pathohistological similarities to the human disease. Preliminary results show that all myelin proteins are decreased, but small doses produce a greater effect on MAG than other potential glycoprotein targets. Monoclonal antibodies that are MAG/SGPG-negative in patients with gammopathy and neuropathy frequently react with ganglioside antigens in nerve. Immunohistological experiments on a patient with mixed axonal and demyelinating symptoms and a monoclonal IgA λ antibody are in progress to establish the relationship of a complex mixture of immunoreactivities to the pathology. In addition to the monoclonal antibody, this patient has polyclonal reactivity to the major LM1 ganglioside of peripheral myelin that is restricted to the IgA class and a low level of IgM reactivity of the MAG/SGPG type. In order to elucidate the functions of acidic glycoproteins in Schwann cell differentiation and myelination as well as to understand mechanisms by which the human anti-glycolipid antibodies may perturb function, we continued our studies of glycolipids in cultured Schwann cells. Basement membrane is well known to be required for Schwann cells to form myelin. We have shown that replacing the usual polylysine substratum with basement membrane (Matrigel), laminin or type IV collagen causes a specific increase in the incorporation of radioactive galactose into the major GM3 ganglioside of the cells and some complex neutral glycosphingolipids, without a similar increase in the synthesis of other lipids, proteins or glycoproteins. The enhanced GM3 synthesis is associated with increased Schwann cell proliferation, and an inhibitor of synthesis of GM3 and the other glycolipids (PDMP) also inhibits basement membrane induced proliferation, suggesting that the increased synthesis may be functionally involved in the proliferative response. The results also show that the Schwann cell response to basement membrane alone is quite different from that which occurs in the presence of neurons, in which case the cells will differentiate and myelinate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02366-17 LMCN
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Receptor-Coupled Adenylylcyclase		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	P. H. Fishman, Ph.D.	Chief, Membrane Biochemistry Section LMCN, NINDS
Others:	M.D. Pak, Ph.D.	Senior Staff Fellow LMCN, NINDS
	X.-M Zhou, M.D., Ph.D.	Visiting Associate LMCN, NINDS
	Z. Wang, M.D., Ph.D.	Visiting Fellow LMCN, NINDS
	P.K. Curran, B.S.	Biologist LMCN, NINDS
	D.E. Kauffman, B.S.	Biologist LMCN, NINDS
	Q.T. Hoang, B.S.	Biologist LMCN, NINDS
COOPERATING UNITS (if any) Jesse Baumgold, Ph.D., Dept. of Radiology, The George Washington University Medical Center, Ronald S. Duman, Ph.D., Division of Molecular Psychiatry, Yale University School of Medicine		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology		
SECTION Membrane Biochemistry Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	6.65	PROFESSIONAL: 3.45 OTHER: 3.2
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The goal of this project is to identify molecular mechanisms involved in the regulation of the β-adrenergic receptor (βAR)-coupled adenylylcyclase. There are three βAR subtypes, β_1AR, β_2AR and β_3AR, that may be regulated differently by agonists and other modulators. One regulatory mechanism is receptor <u>down-regulation</u> whereby cells exposed to agonist exhibit a loss of βAR binding activity with time. We have explored the down-regulation of βAR subtypes in two different cell lines. Rat C6 glioma cells express both β_1AR and β_2AR. When exposed to agonist or forskolin, we observed a coordinate down-regulation of both subtypes. When we quantified the levels of <u>βAR mRNA</u>, a different pattern emerged. In both agonist- and forskolin-treated cells, β_1AR mRNA levels exhibited a biphasic change, initially increasing over 1.5-fold by 1 hr, and then decreasing to 50% of control by 3 hr. The change in β_2AR mRNA levels was monophasic, decreasing with time to <50% of control by 2 hr. Using nuclear run-on analysis, we showed that both the up- and down-regulation of βAR mRNA were due to changes in <u>gene transcription</u> rate. In this regard, the half-life of either βAR mRNA was not substantially altered in the treated cells. When protein synthesis was first blocked, both agonist and forskolin treatment resulted in a 4-fold up-regulation of β_1AR mRNA levels by 6 hr and no down-regulation of β_2AR mRNA. As the genes for both rat βAR subtypes have <u>cAMP responsive elements</u> (CREs), we propose that the up-regulation of β_1AR gene expression is mediated by cAMP-dependent phosphorylation and activation of a CRE-binding protein. By contrast, down-regulation of βAR gene transcription may be mediated by a inducible cAMP early repressor (ICER), a member of the CRE modulatory protein (CREM) family of transcription factors. Human SK-N-MC neurotumor cells express both β_1AR and β_3AR. When the cells were exposed to agonist, there was a down-regulation of β_1AR but not β_3AR binding sites. Interestingly, forskolin treatment did not mediate a down-regulation of β_1AR, and neither treatment caused a down-regulation of β_1AR mRNA levels. As the human β_1AR gene has CREs, these results were unexpected, but support the concept that in addition to differences in regulation among βAR subtypes, there are cell-specific differences. Preliminary studies indicated that ICER was induced by agonist in C6 cells but not SK-N-MC cells. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS 01808-26LMCN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Glycoproteins of Myelin in Development and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.H. Quarles, Ph.D.	Laboratory Chief	LMCN, NINDS	C. Lauter	Chemist
	S.H. Yim, Ph.D.	Special Expert	LMCN, NINDS	J. Hammer	Biologist
	R. Farrer, Ph.D.	Sr. Staff Fellow	LMCN, NINDS		
	Z. Bartoszewicz, Ph.D.	Visiting Associate	LMCN, NINDS		
	A. Chakrabarti, Ph.D.	Sr. Staff Fellow	LMCN, NINDS		
	N. Sasagasako, M.D.	Visiting Fellow	LMCN, NINDS		
	S. Tanner, Ph.D.	IRTA	LMCN, NINDS		
	C. Pizarro, Ph.D.	Sp. Volunteer	LMCN, NINDS		

COOPERATING UNITS (if any)

Dept. Neuroscience, Cleveland Clinic Foundation, Cleveland, OH

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Myelin and Brain Development Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

7.35

PROFESSIONAL:

5.8

OTHER:

1.55

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The myelin-associated glycoprotein (MAG) is a member of the immunoglobulin gene superfamily that is localized in the periaxonal membranes of PNS and CNS myelin sheaths where it functions in glia-axon interactions and may be involved in transmitting signals from axons to myelin-forming cells. It occurs in two developmentally regulated isoforms (L- and S-MAG) with differing C-terminal tails generated by alternative splicing of its mRNA. The carbohydrate in MAG consists of a mixture of oligosaccharides, many of which are sialylated and sulfated and are currently being characterized. This year, we extended our previous studies on the abnormal expression of MAG isoforms and their glycosylation in dysmyelinating mice to trembler mutants that exhibit abnormalities of myelin restricted to the PNS. The relative expression of MAG isoforms was normal in the nerves of these mice, but PNS MAG contained increased α 2-3 linked sialic acid and galactose that may contribute to the nerve pathology. In addition to our studies of MAG, a novel 80kDa glycoprotein in PNS and CNS myelin containing primarily mannose-rich oligosaccharides was identified and is being characterized. The expression of MAG in cultured oligodendrocytes and Schwann cells continues to be studied to identify factors controlling its expression and probing its function in glia-axon interactions and transmembrane signaling. Recent experiments with immortalized Schwann cell lines indicate that expression of MAG and other myelin proteins are up-regulated independently by decreased cellular proliferation and increased cell to cell contact. Although the control seems to be at the level of messenger RNAs, it is not associated with changes in cAMP levels, nor do nuclear runoff assays indicate it is at the transcriptional level. Experiments on the tyrosine phosphorylation of growth factor receptors in oligodendrocyte progenitors continued to determine whether the mechanism by which GM3 ganglioside enhances the expression of myelin components involves receptor modulation. Although these experiments have not so far uncovered a mechanism for the effect of GM3, they revealed a dramatic downstream effect of PDGF and FGF on the MAP kinase in these cells. Also our investigation of gangliosides in oligodendrocyte progenitors has shown that the A2B5 antibody, which is widely used to identify these cells, reacts most strongly with a novel ganglioside that is currently being characterized. Finally experiments to identify and characterize the putative MAG receptor in axonal surface membranes has been undertaken utilizing purified axolemma and the MAG-expressing lines of oligodendrocytes and Schwann cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01309-30
LMCN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. H. Fishman, Ph.D.	Chief, Membrane Biochemistry Section	LMCN, NINDS
Others:	P. A. Orlandi, Ph.D.	Research Associate	LMCN, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Membrane Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	1.45	PROFESSIONAL:	1.3	OTHER:	0.15
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cholera toxin (CT) produced by *Vibrio cholerae* is the causative agent of the disease cholera. CT consists of a B subunit that binds to ganglioside G_{M1} on the surface of the intestinal mucosal cell and an A subunit that is involved in activation of adenylyl cyclase. The latter process requires that the A subunit be reduced to generate the A_1 peptide. The A_1 peptide is an ADP-ribosyltransferase that catalyzes the transfer of ADP-ribose from NAD^+ to the α subunit of the stimulatory G protein (G_s) of adenylyl cyclase. This modification blocks the intrinsic GTPase activity of G_s and keeps the cyclase in a persistently activated state. We have been investigating the detailed mechanism of cellular processing and activation of CT. As a model, we are using human intestinal CaCo-2 cells, which behave in culture as differentiated enterocytes, the natural target for CT. We are particularly interested in events during the lag period between toxin binding and cyclase activation. We previously have shown that the holotoxin binds to the cell surface with the A subunit facing away from the membrane and that the holotoxin is internalized during the lag period. At the end of the lag period small amounts of A_1 peptide are generated by the cells, and the cyclase becomes activated. We now show that CT is reduced by a cellular reductase activity which we have identified as protein disulfide isomerase (PDI). Although PDI is found in the lumen of the endoplasmic reticulum, some is present on the cell surface. Using cell membrane-impermeant inhibitors, we found that the latter pool of PDI is not involved in CT reduction, and thus generation of A_1 occurs at an intracellular site. It has been proposed that CT is internalized through non-coated invaginations on the plasma membrane known as caveolae. These microdomains are enriched in cholesterol, glycolipids, glycolipid-anchored proteins, and are resistant to nonionic detergents. To explore this possibility, we exposed CaCo-2 cells to the cholesterol-binding drug filipin, which has been reported to perturb caveolae and their function. We observed that filipin treatment blocks the ability of CT to activate adenylyl cyclase and the effect is rapid, dose-dependent and reversible. Furthermore, no A_1 peptide is formed in filipin-treated cells. Taken together, our results support a model in which CT enters the cell through caveolae, and undergoes reduction by PDI in an intracellular compartment to generate the A_1 peptide.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02915-01
LMMN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Viral Vectors for Gene Transfer into Glial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: C. S. Tornatore, M.D. Acting Section Chief LMMN

Others: S. Keir, Ph.D. IRTA Fellow LMMN
R. Hamilton, B.S. Biologist LMMN
K. Meyers Biol. Lab. Techn. LMMN

COOPERATING UNITS (if any)

Hematology Branch, NHLBI; Developmental and Metabolic Neurology Branch, NINDS

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Molecular Therapeutics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF HOURS: 1.95 PROFESSIONAL: 1.2 OTHER: 0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1) Evaluation of Adeno-Associated Virus (AAV-2) as a Vector for Gene Transfer into Cells of Glial Origin: AAV-2 is single-stranded DNA parvovirus which has recently emerged as potentially useful tool for gene transfer, both *ex vivo* and *in vivo*. To determine whether AAV-2 could be used for gene transfer into glial cells, both primary glial cells (100% GFAP positive) and a glial cell line (SVG) were transduced with a Lac-z expressing AAV-2 recombinant. Using an MOI of between 1-10%, 95% of the primary glial cells were transduced and 99% of the glial cell line was transduced. To determine whether a functional protein could be effectively transferred, the SVG cell line was transduced with a human CD4 expressing AAV-2 recombinant. A stable CD4 expressing SVG cell line was easily established as determined by immunohistochemistry and Western blotting for CD4. To determine whether this CD4 is functional, the SVG-CD4 cells were infected with HIV-1 (IIb strain). Viral replication and the number of infected cells was ten-fold higher in the SVG-CD4 cells than in the parental SVG cell line, consistent with the expression of functional CD4 protein.

2) Development of Chimeric JCV Vector for Gene Transfer into Cells of Glial Origin: JC virus is a human polyoma virus which has a glial-specific promoter. As such, a recombinant JCV could also be a useful tool for glial specific gene transfer. We have already constructed two different viral chimeras in which the early portion of the viral genome (the large T protein gene) has been replaced with a cDNA marker gene (either tyrosine hydroxylase or green fluorescent protein). In addition constructs have been made which have one of three different JCV promoters (Mad1, Mad4, and Mad8). When the chimeric DNA is transfected into cells which do not express T protein, these constructs have been found to express the marker gene, but fail to replicate. However, when these constructs were transfected into a cell line (SVG) which complements the chimera by producing T protein, the SVGs act as a packaging cell line and produce high titer recombinant JCV. This recombinant virus has been used to infect primary human fetal glial cells and has been found to efficiently express the marker gene TH. Southern blots of DNA extracted from the infected cultures confirmed that the viral genome was the chimeric construct. Thus, a recombinant JCV particle has been packaged, and a novel packaging cell system has been established.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02907-02
LMMN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Multitarget-Ribozymes as Analytical and Therapeutical Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: M. Schubert, Ph.D. Section Chief LMMN

Others: S.-Y Paik, Ph.D., Ph.D. Visiting Fellow LMMN
B. Lewis Lab. Technician LMMN

COOPERATING UNITS (if any)

William J. Mitchell, Laboratory of Experimental Neuropathology, NINDS; Richard Morgan, Clinical Gene Therapy Branch, NCHGR.

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Molecular and Viral Genetics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF HOURS: 0.95 PROFESSIONAL: 0.7 OTHER: 0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ribozymes are catalytic RNAs that can be targeted to specifically cleave selected RNAs. Such target RNAs could be viral RNAs or cellular RNAs. One of the goals of this study was to develop ribozymes against HIV-1 as part of a gene therapy in AIDS patients. Our second goal is to develop ribozymes for specific cellular mRNAs in the CNS. A specific combination of ribozyme target sites together with specific promoters for ribozyme expression could be employed in a reverse genetic approach in transgenic mice. Our plan is to generate specific phenotypes in transgenic mice to cause neuronal dysfunction by multitarget-ribozymes in order to study neuronal function. We have previously developed multitarget-ribozymes that cleave HIV-1 RNA at up to nine different sites, and protect cells from HIV-1 pathogenesis in tissue culture. Our data suggest that multitarget-ribozymes are functional in cell nuclei and they cleave unspliced HIV-1 RNA. In addition, we found that the same multitarget-ribozyme transcript can be translated in the cytoplasm. This strongly suggests that a highly effective negative transdominant mutant HIV-1 Rev protein could be expressed from the same mRNA that also functions as a multitarget-ribozyme. We are currently recloning our HIV-1 multitarget-ribozyme for insertion into a retrovirus vector (MMLV). If the combination of multitarget-ribozyme and transdominant negative HIV-1 Rev protein increases antiviral efficacy against HIV-1, these vector may be included in clinical trials with HIV-1 infected and uninfected twins. For our initial studies of multitarget-ribozyme effectiveness in transgenic mice, we have recently synthesized two octaribozymes, one against the ICP4 protein mRNA of herpes simplex virus and the other against the beta-galactosidase protein mRNA. The efficacy of these ribozymes will initially be tested in tissue culture and subsequently in transgenic mice. The purpose of these studies will be to rule out toxicity of multitarget-ribozymes in vivo. Secondly, these transgenic mice may resist herpes virus neuropathogenesis because of the multitarget-ribozyme. The octaribozyme against the beta-galactosidase will be used to inhibit beta-galactosidase expression in select neurons of the hippocampus of already existing transgenic mice. If multitarget-ribozymes are highly effective in vivo, they could complement gene knock-out experiments for the generation of mouse models for neuronal dysfunction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02904-02 LMMN
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Molecular and Cellular Neurologic Therapeutics		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	C. S. Tornatore, M.D.	Acting Section Chief LMMN
Others:	B. Cairns Baker, Ph.D.	IRTA Fellow LMMN
	R. Hamilton, B.S.	Biologist LMMN
	K. Meyers	Biol. Lab. Techn. LMMN
COOPERATING UNITS (if any) Pre-Clinical Neuroscience Section, NIMH		
LAB/BRANCH Laboratory of Molecular Medicine and Neuroscience		
SECTION Molecular Therapeutics Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF HOUR:	2.25	PROFESSIONAL: 1.5 OTHER: 0.75
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The grafting of fetal tissue in the treatment of neurodegenerative disorders would be greatly facilitated if a viable human fetal cell line could be substituted for primary fetal tissue. A permanent, <u>immortalized human fetal astrocyte cell line</u> (SVG) has been established which maintains the phenotypic characteristics of fetal astrocytes. The cDNA for human tyrosine hydroxylase, type 2, was stably transfected into the SVG cell line, establishing a second cell line, SVG-TH. These cells have continuously expressed TH for the past eighteen months, with no appreciable change over time. HPLC analysis of the supernatant from these cells consistently found 4-6 pmol/ml/min of L-dopa produced but only if BH4 was added to the media. Unexpectedly, the SVG-TH were also found to secrete serotonin, which was not found in the parent SVG cells. To determine the viability of these cells in vivo, SVG-TH cells were grafted into the normal striatum of Sprague-Dawley rats and followed over time. A panel of antibodies were used to unequivocally differentiate the engrafted cells from the host parenchyma, including antibodies to: SV40 large T antigen (expressed in the SVG-TH cells), human and rat MHC class I, vimentin, GFAP, serotonin and tyrosine hydroxylase. While the graft was easily identified with the first week, over the course of a four week period of time the engrafted cells decreased in number. Concomitantly, rat MHC class I, class II, CD4, and CD8 expression in the vicinity of the graft increased, consistent with xenograft rejection. When the SVG-TH cells were grafted to the lesioned striatum of 6-hydroxydopamine-treated rats, rotational behavior of the rat decreased by 50% initially, then slowly returned to baseline over the next four weeks, paralleling graft rejection. In contrast, the SVG cell line has been successfully engrafted and survived in the striatum of the rhesus macaque for up to 9 months, immunologically acting as an allograft. MPTP-lesioned macaques are currently being characterized for subsequent engraftment with the SVG-TH cell line. We have also constructed mammalian expression vectors which express either wild type SV40 T protein or the first 155 amino acids of the N-terminus of SV40 T protein to determine whether sub-domains of the oncoprotein are capable of immortalizing primary cells without altering the phenotypic properties of the primary cells. </p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02908-02 LMMN
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Gene Delivery to Nondividing Cells of the CNS		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
P.I.:	M. Schubert, Ph.D.	Section Chief LMMN
Others:	G. G. Harmison, II, M.S.	Chemist LMMN
	S.-Y Paik, Ph.D., Ph.D.	Visiting Fellow LMMN
	B. Lewis	Lab. Technician LMMN
COOPERATING UNITS <small>(if any)</small> S. Karlsson, Section Chief, and J. Reiser, Visiting Scientist, Developmental and Metabolic Neurology Branch, NINDS.		
LAB/BRANCH Laboratory of Molecular Medicine and Neuroscience		
SECTION Molecular and Viral Genetics Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL Staff Hours:	0.8	PROFESSIONAL: 0.15 OTHER: 0.65
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>Currently, there is no <u>viral vector</u> available that allows the stable integration and expression of genes in <u>postmitotic cells</u>. As a lentivirus, HIV-1 is able to transport its preintegration complex through the nuclear pores and to integrate into nondividing cells like macrophages or dendritic cells. One of the goals of this study is to develop safe defective HIV-1 particles that could function as a <u>gene delivery</u> and <u>expression vector</u> for postmitotic cells.</p> <p>We have earlier developed a <u>defective HIV-1 helper virus</u> construct, <u>HDPack1</u>. This construct was now used to generate <u>HIV-1 particles</u> that contained either the <u>ecotropic</u> or <u>amphotropic</u> <u>Env proteins</u> of <u>Moloney murine leukemia virus</u>. The genomic RNA of these particles consisted of a <u>mini-HIV-1 RNA</u> encoding a <u>neomycin resistance gene</u>. These <u>pseudotype HIV-1</u> particles were able to infect other species such as mouse cells and confer neomycin resistance to these cells. These results demonstrated for the first time that all functions and structural elements necessary for the generation of the defective HIV-1 vector were properly provided by our helper virus DNA construct. This construct will now be used for the generation of an <u>expression vector</u> for postmitotic cells.</p> <p>Several attempts to isolate a <u>stable cell line</u> that constitutively sheds defective HIV-1 particles were not successful. A single cell clone was isolated but it produced low levels of virus particles for only five weeks. It has recently been described that the <u>Vpr protein</u> of HIV-1 which is encoded by HDPack1 resulted in very high virus titers, suggesting that HDPack1 is not toxic but that a clonal expansion to a cell line may not be possible with Vpr present. Since transient transfections of HDPack1 in 293 cells have yielded up to 10⁸ HIV-1 like particles per ml, we will try to use this transient system to generate sufficient amounts of the viral expression vector.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02851-04
LMMN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HIV-1 Infection in Human Fetal Brain Cell Cultures and Pediatric AIDS Brain Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	E. O. Major, Ph.D.	Chief	LMMN	
Others:	U. Ahmed	Summer Student	LMMN	C. Monaco Guest Worker,
	W. Atwood, Ph.D.	Senior Staff Felloow	LMMN	Bologna, Italy
	G. Brashears	Biologist	LMMN	
	K. Conant, M. D.	Senior Staff Fellow	LMMN	
	M. Gravell, Ph.D.	Microbiologist	LMMN	
	J. Hou	Co-op	LMMN	

COOPERATING UNITS (if any)

A. Facchini, Univ. Bologna, Italy; A. Degrassi, Udine Univ. Sch. Med., Italy; S. Houff, VA Hosp., Washington, D. C.; R. Youle, SNB, NINDS and F. Chiodi, Karolinska Institute, Sweden

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Section on Molecular Medicine and Virology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF HOURS:

4.05

PROFESSIONAL:

2.85

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have established an in vitro model of latent HIV-1 infection in human fetal astrocytes. Several weeks following infection or transfection, cocultivation with uninfected lymphocytes or stimulation with the cytokines TNF-alpha and IL1-beta will increase viral production from this cell type. We have demonstrated that phorbol 12-myristate 13-acetate (PMA) also increases HIV-1 p24 production from the primary human astrocyte. Using electrophoretic mobility shift assay (EMSA) in combination with supershift studies using specific antibodies, we demonstrated that PMA, like TNF-alpha increases the p50/p65 form of NF-kB. Furthermore, we also showed that the protein kinase inhibitor H7 inhibits PMA and TNF-alpha associated increases in HIV-1 expression at a time when it has little to no inhibitory effect on the associated increases in p50/p65 NF-kB. Thus, unless p50/p65 NF-kB or its binding is affected by H7 in a manner that cannot be resolved by EMSA, an increase in this form of NF-kB is not always sufficient to increase HIV-1 expression from the astrocyte.

The laboratory is also investigating the ability of specific RNases to inhibit the multiplication of HIV-1 in lymphocyte cell lines. Onconase and bovine seminal RNase were shown to block infection of HIV-1 in productivity infected cell lines. This block appears from an intracellular mechanism of RNase activity.

In addition to the regulation of NF-kB in infected astrocyte cultures, the viral rev protein also seems to be a target for cellular control. Using EMSA assays, astrocytes produce a factor which binds the RRE-rev complex. This factor also responds to cytokine and PMA stimulation. Both the NF-kB and the rev binding factor appear to be limited in concentration compared with cells highly susceptible to HIV-1 infection. These data point to a mechanism of HIV-1 latent infection in brain involving reduced levels of cellular factors necessary for productive HIV-1 multiplication. The role of the astrocyte as a reservoir of HIV-1 in the brain is being investigated using the in vitro model of infection.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02852-04
LMMN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurogenesis and Gliogenesis in the Developing Human Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	E. O. Major, Ph.D.	Chief	LMMN
Others:	W. Atwood, Ph.D.	Senior Staff Felloow	LMMN
	E. Dayton, Ph.D.	Special Expert	LMMN
	J. Staehli	Special Volunteer	LMMN
	R. Traub, B.S.	Microbiologist	LMMN

COOPERATING UNITS (if any)

Peter G. E. Kennedy, Department of Neurology, Glasgow University, Scotland

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Section on Molecular Medicine and Virology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF HOURS:

1.5

PROFESSIONAL:

1.15

OTHER:

0.35

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The NF-1 and AP-1 transcription factors have adjacent binding sites in the regulatory region of a number of genes. Because of the possible involvement of NF-1 with the expression of genes expressed in the CNS/PNS, we have screened cDNA libraries prepared from human neonatal and fetal brain tissue for the possible presence of a brain specific NF-1 factor with an oligonucleotide probe homologous with the DNA binding domain of the NF-1. Two clones from the neonatal brain library and one clone from the fetal brain library were sequenced. The 3 sequenced cDNA clones were homologous with each other, except for a few bases at the 5' end of the two longest clones and a 150-bp insertion in the 3'-region of the shortest clone. The DNA binding region located at the 5'-end of the clones was highly conserved between the brain clones and that reported for the HeLa NF-1 clone. On the other hand, the 3'-region of the c-DNA clones isolated from the brain libraries were highly homologous but differed from that reported from HeLa cells. The 3'-region of the NF-1 molecules were reported to contain the transcriptional activational domain of the molecular. One of the brain cDNA clones was cloned into a T7 RNA polymerase expression vector, and a specific size protein was overproduced on induction of expression with IPTG. An extract prepared from cells overproducing the specific protein was demonstrated to contain specific binding activity. In order to determine if other classes of NF-1 could be detected in primary fetal brain cells, RT-PCR analysis was performed with poly A-selected RNA from primary fetal cells and HeLa cells. With the use of class-specific primers the expression of at least four classes of the NF-1 protein family could be detected in both the brain and the HeLa cell lines. In the fetal brain cultures, however, one clone, NF-1/AT1 was highly expressed compared with the other 3 classes. Astrocyte cultures were also analyzed for expression of arachidonic acid metabolites in response to cytokine stimulation. In the presence of IL-1 beta, human astrocytes produce prostaglandin E₂ and prostaglandin F₂ alpha. Other intermediate compounds in the cyclooxygenase pathway are not made unlike similar cultures of rodent astrocytes. Both of these studies highlight the fact that human astrocytes have phenotypic and genotypic differences compared with rodent models of neuro and gliogenesis.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02818-06
LMMN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pseudotypic Defective Interfering HIV Particles as an Antiviral Therapy for AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: M. Schubert, Ph.D. Section Chief LMMN

Others:	G. G. Harmison II, M.S.	Chemist	LMMN
	S.-Y Paik, Ph.D., Ph.D.	Visiting Fellow	LMMN
	Z. Ye, Ph.D.	Senior Staff Fellow	LMMN
	A. C. Banerjea, Ph.D.	Senior Staff Fellow	LMMN
	B. Lewis	Lab. Technician	LMMN

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Molecular and Viral Genetics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL Staff Hours:

1.95

PROFESSIONAL:

1.1

OTHER:

0.85

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to develop defective interfering HIV-1 particles that are able to target HIV-1 expressing cells and to interfere with HIV-1 replication in these cells. These particles would use wild type virus as a source for all structural proteins to generate new defective viruses. These in turn will then repeat this cycle in other HIV-1 infected cells. The overall goal is to reduce the HIV-1 load in the patient over a long period of time and thereby delay the onset of AIDS. Many complex elements of this antiviral strategy must be studied in detail at the molecular level. In the past, we have developed defective interfering HIV-1 proviral DNAs that interfere quite effectively with wild type virus in tissue culture.

During the past year, we have constructed several new candidate defective interfering proviral DNAs. We are in the process of coexpressing these DNAs with wild type HIV-1 DNA to demonstrate for the first time a transfer of the interfering genes through infection by defective interfering HIV-1 particles. For this purpose, a hygromycin resistance gene was inserted into the defective HIV-1 provirus under control of a TK promoter. Expression of this resistance gene will be independent of HIV-1 Tat protein. Transfer of the resistance and the interfering genes through infection, however, will be totally dependent on the structural proteins provided by wild type HIV-1. For the future targeting of these defective interfering HIV-1 particles through pseudotype virus formation, we have synthesized several new candidate chimeric CD4 proteins. These proteins will be evaluated with respect to their efficiency of expression, transport to the cell surface, insertion into HIV-1 particles and subsequent binding of these particles to HIV-1 Env expressing cells, followed by infection of these cells and defective interfering HIV-1 proviral insertion. These studies will be most important to understand pseudotype virus formation and the development of targeted viral vectors for postmitotic cells of the CNS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS02791-07
LMMN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Replication and Pathogenesis of Enveloped Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: M. Schubert, Ph.D. Section Chief LMMN

Others: S.-Y Paik, Ph.D., Ph.D. Visiting Fellow LMMN
Z. Ye, Ph.D. Senior Staff Fellow LMMN
B. Lewis Lab. Technician LMMN

COOPERATING UNITS (if any)

K. Ozato, Laboratory of Molecular Growth Regulation, NICHD, NIH, Bethesda, MD; R. R. Wagner, University of Virginia, Charlottesville, VA; Volker ter Meulen, University of Wurzburg, Germany.

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Molecular and Viral Genetics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF HOURS

0.9

PROFESSIONAL:

0.65

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously shown that the vesicular stomatitis virus (VSV) matrix protein plays a key role in viral pathogenesis by inhibiting gene expression from chromosomal DNA and by disrupting the cytoskeleton. Infections with the VSV related measles virus can in some cases lead to persistent infections of the CNS. It has been reported that during persistent infections the measles virus matrix gene has accumulated a high number of U to C transitional mutations. The reason for this is unclear. It may be linked to a potential cytotoxicity of the wild type matrix protein that must be neutralized to establish and maintain a persistent infection. We have obtained a total of three wild type matrix genes and ten mutant measles matrix genes isolated from patients with subacute sclerosing panencephalitis (SSPE) and with measles virus inclusion bodies encephalitis (MIBE). These genes were cloned under control of cytomegalovirus promoter for expression in eukaryotic cells. Experiments are in progress to study the subcellular localization of these proteins as well as any potential cytopathic effects that may explain why these mutant genes are selected during these fatal persistent measles virus infections of the CNS.

In collaboration with Dr. Ozato, it was found that VSV infections induce a new nuclear DNA binding factor that binds to the interferon-stimulated response element. This factor is most likely involved in interferon induction. The presence of the factor depends on viral transcription. It can also be induced by transfection of double stranded RNA. VSV is very sensitive to antiviral effects of interferon. In fact, the shutoff of cellular transcription by VSV matrix protein may counteract the effect of interferon. This may in part explain the interferon induction and suppression phenotypes of the virus. These findings have important implications for our understanding of viral pathogenesis and disease progression.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01983-24

LMMN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Pathogenesis of JC Virus-Induced Progressive Multifocal Leukoencephalopathy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	E. O. Major, Ph.D.	Chief	LMMN		
Others:	W. Atwood, Ph.D.	Senior Staff Fellow	LMMN	R. Traub, B.S.	Microbiologist LMMN
	G. Ault	Sr. Staff Fellow	LMMN	T. Shinohara, Ph.D.	Spec.Vol. LMMN
	B. Curfman	Microbiologist	LMMN		
	L. Durham, M. S.	Biologist	LMMN		
	J. Hou	Co-op	LMMN		
	M. Monaco, Ph.D.	Special Volunteer	Bologna, Italy		

COOPERATING UNITS (if any)

AIDS Clinical Trial Group, OAR, OD, NIH, Thomas Weber, M.D., Department of Neurology, George-August-Universitat, Gottingen, Germany

LAB/BRANCH

Laboratory of Molecular Medicine and Neuroscience

SECTION

Section on Molecular Medicine and Virology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF HOURS:

6.28

PROFESSIONAL:

3.55

OTHER:

2.73

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The laboratory has participated in a Phase II clinical trial for treatment of AIDS patients with the demyelinating disease, PML. We had previously demonstrated the effectiveness of the nucleotide analogue, cytosine arabinoside (ARA-C) to block replication of the viral DNA. Concentrations of ARA-C used were not toxic to the human glial cells in culture. Currently 38 AIDS patients with biopsy proven PML (the laboratory confirms the diagnosis using in situ DNA hybridization to detect viral DNA) have been enrolled in the study. We have examined the peripheral blood and cerebrospinal fluid from these patients as treatment proceeds. In almost all cases, viral DNA has been found in the blood. In several samples, the viral DNA was identified in the B lymphocyte population and not T cells. This observation is consistent with previous clinical samples of B cell infection in bone marrow and spleen. The treatment protocol will continue until a maximum of 90 patients are enrolled or two years have elapsed. It is too early in the study to determine efficacy of the drug.

Molecular biology studies are now linking B and glial cell susceptibility to JCV infection at the transcriptional level. A member of the transcription factor family, NF-1, appears to be highly expressed in cells in which JCV can replicate. The brain specific NF-1/AT1 is also found by Northern blot analysis in B cell lines that allow JCV to multiply. Further analysis of the NF-1/AT1 clone transfected into non-permissive cells is being done. The NF-1 factor, however, is not increased in its activity with cytokine stimulation using TNF-alpha or IL-1 beta. These cytokines stimulate the NF-kB transcription factor which is essential for HIV-1 multiplication in glial cells. It is not likely that JCV uses NF-kB for transcription nor is it likely that JCV infection is augmented by cytokines.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02899-02 LNLC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural Mechanisms Controlling Breathing in Mammals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and Institute affiliation)

PI: J.C. Smith, Ph.D.

Research Physiologist

LNLC, NINDS

Others:

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ Human tissues☒ Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to provide information on basic neural mechanisms involved in the generation and control of respiratory movements in mammals. The long-range goal is to explain the ontogeny and neurogenesis of respiratory movements in terms of the molecular, biophysical, synaptic, and network properties of respiratory neurons in the mammalian brainstem and spinal cord. Current work focuses on cellular and network mechanisms generating the respiratory rhythm in the brainstem. A set of interrelated, multidisciplinary studies are ongoing to determine: sites, cellular components, and architecture of brainstem networks involved in generation and transmission of respiratory rhythm; biophysical properties and synaptic interactions of rhythm-generating neurons; and neurochemical mechanisms for modulation and synaptic transmission of rhythm. Experiments are performed with isolated in vitro brainstem-spinal cord and brainstem slice preparations from fetal, neonatal, and juvenile rodents. The critical brainstem locus containing the populations of neurons generating the rhythm has been identified. Novel in vitro slice preparations containing this critical region and functionally active respiratory networks have been developed and are used for experimental analysis of mechanisms concurrently at cellular and network levels. Computational approaches are being used in parallel to experimental studies to model respiratory neurons and networks. A first generation of computational models of the respiratory oscillator have been developed, based on available data of neuron biophysical properties and network architecture in the neonatal and adult brainstem. Simulations with these models are able to mimic many features of the oscillatory behavior of brainstem respiratory neurons found with intracellular recordings in the neonatal system in vitro and the adult nervous system in vivo. These models are currently being applied to explore principles of design and control of the respiratory oscillator at different stages of nervous system development.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02857-03 LNLC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Feasibility Study of an Intracortical Visual Prosthetic Device for the Blind

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and Institute affiliation)

PI:	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLC, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	A. Reina	Summer Student	LNLC, NINDS

COOPERATING UNITS (if any)

Fundamental Neurosciences Program, NINDS (W.J. Heetderks and F.T. Hambrecht); Surgical Neurology Branch, NINDS (C.V. Kufta); Instrumentation and Computer Section, BNP, DIR, NINDS (B. Smith, Chief)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.4

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- | | | |
|--|--|----------------------------------|
| <input checked="" type="checkbox"/> (a) Human subjects | <input type="checkbox"/> Human tissues | <input type="checkbox"/> Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input checked="" type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to evaluate the feasibility of a visual prosthesis for totally blind individuals by stimulating chronically implanted microelectrodes in the visual cortex. As reported last year, a 42 year old woman who has been blind for 22 years was implanted with an array of 38 electrodes in the visual cortex. Stimulation of individual electrodes produced sensations of light called phosphenes. The results suggest that it may be possible to produce a useful visual prosthesis if more electrodes are implanted. A new protocol for implanting up to 256 microelectrodes in the visual cortex has been approved by the Institute's Human Review Board. A 256-channel, microprocessor-controlled stimulation system has been constructed and is now undergoing final evaluation prior to testing with animals. A camera interface that will allow stimulation of the electrode array with visual images is nearing completion. A new laser system can remove Parylene-C insulation from the electrodes and provide a much better tip exposure than has been possible with any other technique. The new head-mounted, multicontact connector, developed in conjunction with PI Medical, is undergoing final bench testing before animal implantation. The next patient should be implanted during FY 1995.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02787-07 LNLC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Network Function in the Developing Spinal Cord of the Chick Embryo.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

PI:	M.J. O'Donovan, M.B.,Ch.B.,	Research Physiologist	LNLC, NINDS
Others:	Ph.D.		
	N. Chub, Ph.D.	Fogarty Fellow	LNLC, NINDS
	A. Donevan, Ph.D.	Fogarty Fellow	LNLC, NINDS
	Peter Wenner, Ph.D.	Irta Fellow	LNLC, NINDS
	Patt Carr, Ph.D.	Special Volunteer	LNLC, NINDS
	Uri Cohen	Howard Hughes Scholar	LNLC, NINDS

COOPERATING UNITS (if any)

Dept. Physiol., Yale University, New Haven, CT (L. Cohen); Dept. Anat., Hebrew Univ., Jerusalem, Israel (A. Lev-Tov)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.2

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> Human tissues | <input checked="" type="checkbox"/> Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is concerned with analyzing the development and function of spinal networks in the spinal cord of the chick embryo. One focus is the synaptic organization of the lumbosacral-cord. A second interest is in the cellular and network mechanisms responsible for the genesis of spontaneous network activity. A third interest is in establishing the function of spontaneous neural activity during development with particular reference to the control of gene expression. All experiments are performed on an isolated preparation of the spinal cord which is maintained *in vitro*.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02254-19 LNLC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Repair of Injured Nervous Tissue with Foreign Grafts

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and Institute affiliation)

PI:	A.A. Zalewski, M.D.	Section Chief	LNLC, NINDS
Others:	N.A. Azzam, Ph.D.	Biologist	LNLC, NINDS
	R.N. Azzam	Biologist	LNLC, NINDS
	J.D. Ziemnowicz	NIH Special Volunteer	LNLC, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Neuronal Regeneration Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.4

PROFESSIONAL:

2.0

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ Human tissues ☒ Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Peripheral nerve grafting is an important clinical procedure for the restoration of nerve function after trauma that creates a gap between injured nerve ends. We have previously reported that nerve allografts can be used to repair a long gap in a nerve, but only if the recipient is chronically immunosuppressed with the drug Cyclosporin A (Cy-A). Cy-A therapy cannot be stopped since nerve rejection occurs and host axons that traverse the graft degenerate. It is puzzling why kidney and heart allografts survive indefinitely after stopping Cy-A treatment whereas nerve allografts are rejected. In order to help resolve this problem we performed an experiment with nerve allografts in which we used the same Cy-A treatment protocol and rat strains used by others to induce permanent survival of heart allografts. DA peroneal nerve grafts (4 cm long) were bilaterally grafted into the legs of PVG recipients that were given Cy-A (15 mg/kg, intramuscularly) according to the following schedule: Cy-A for 1 week beginning the day of surgery followed by 2 weeks off the drug, then another week of treatment and a second two weeks off Cy-A and finally by a 3 day boost of Cy-A with no further treatment. We found that untreated PVG rats rejected DA nerve grafts by 28 days. On the other hand, DA nerves survived in Cy-A-treated PVG recipients at 63 days, the last period of observation. Since only one DA nerve was removed from various immunosuppressed PVG rats, we plan to wait until 100 days and regraft these rats with a new DA or ACI rat nerves. If the Cy-A treatment described induces donor-specific graft tolerance (i.e., long-term graft acceptance), as proposed by others, then the second as well as the original DA nerves should survive whereas the ACI graft should be rejected. We are encouraged to believe that for the first time a model of tolerance induction to nerve allografts will be achieved with short-term drug immunosuppression. In another experiment we used the electron microscope to determine the long-term fate of Schwann cell basement membrane (SCBM) in rejected nerve allografts. Some investigators believe that SCBM tubes alone might support host nerve fiber growth. This notion assumes that SCBM persists as tubes indefinitely. We found that SCBM collapsed and became fragmented at 4 weeks, a time at which all allogeneic cells were rejected. From 4-8 weeks postoperatively, SCBM gradually disappeared or remained as small irregular patches. Moreover, host axons and Schwann cells grew only 1-2 cm into the 6 cm long allografts. The degradation of SCBM tubes implies that only a short gap in an injured nerve can be repaired with an acellular graft.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02160-21 LNLC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intrinsic Properties of Motor Units

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and Institute affiliation)

PI:	R.E. Burke, M.D.	Chief	LNLC, NINDS
Others:	W.B. Marks, Ph.D.	Research Physiologist	LNLC, NINDS
	B. Ulfhake, M.D., Ph.D.	Assoc. Professor	Karolinska Institutet
	R. E. W. Fyffe, Ph.D.	Assoc. Professor	Wright State Univ
	W. Cameron, Ph.D.	Assoc. Professor	Univ. Pittsburgh
	J. Nguyenkim	NIH Summer Student	Univ. Oklahoma

COOPERATING UNITS (if any)

Dept. of Anatomy, Karolinska Institutet, Stockholm, Sweden; Dept. of Anatomy, Wright State University, Dayton, OH; Dept. Behav. Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.1

PROFESSIONAL:

0.7

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> Human tissues | <input checked="" type="checkbox"/> Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to provide information about the populations of motor units that make up large limb muscles in mammals. Recent work has concentrated on the electrophysiological and morphological characteristics of spinal cord motoneurons, particularly on neuroanatomic studies and computer modeling of individual, functionally-identified motoneurons. We have attempted to identify the fundamental factors that control dendritic morphology by developing a relatively simple stochastic (Monte Carlo) model that can reproduce a wide range of statistical properties of actual motoneuron dendrites. This approach is being used to compare the fundamental dendritic structure of several groups of cat motoneurons and interneurons, as well as the morphologies of dendrites in two groups of motoneurons during postnatal development. We are extending these studies to examine the quantitative characteristics of dendritic trees in three dimensions, in order to explore whether the 3D anatomy of dendrites results, at least in part, from factors intrinsic to the dendrites themselves. Our dendritic simulations take account only of intrinsic factors. These simulations and the data that underlie them are also being used to test ideas about whether dendritic morphologies are optimized for particular functions while minimizing factors that can be regarded as biological costs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02079-22 LNLC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Models of Neurophysiological Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and Institute affiliation)

PI:	W.B. Marks, Ph.D.	Research Physiologist	LNLC, NINDS
Others:	R.E. Burke, M.D.	Chief	LNLC, NINDS
	M.J. O'Donovan, M.B.Ch.B., Ph.D.	Research Physiologist	LNLC, NINDS
	T.G. Smith, Ph.D.	Research Physiologist	LNP, NINDS

COOPERATING UNITS (if any)

Lab. of Neurophysiology, NINDS (T.G. Smith)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

0.7

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ Human tissues ☒ Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In last year's Annual Report we described our search for a function of the shape of motoneuronal dendrites which increases for shapes more closely resembling natural dendrites. This would suggest a "goal" for the dendrite. To test this, model dendrites are compared to natural ones. The models are free to vary in branch lengths, diameters and angles, and branching pattern, but are constrained to natural rates of taper and the electrical properties of the membrane. We concluded that the shape function F should depend on the extracellular volume accessed. Local volumes within a distance R of the dendrite were weighted by the electrotonic coupling to the soma of the dendrite nearpoint, and summed to give weighted extracellular volume V . This was divided by its "cost", dendrite volume v , to give the shape function $F = V/v$. F was greater for branching structures, greater for dendrites which branch more near the soma, a property of motoneurons, and greater when the structures had approximately natural extent. This year we developed an efficient algorithm to search for optimum dendrites having largest F by varying the lengths, diameters and angles of all their branches. The lengths of branches of optimized model dendrites at successive branchpoints were similar to the natural lengths. The low order branches are shorter perhaps because they are thicker and cost more in volume. A plot of F against v for natural dendrites revealed that for small v they do not access the maximum possible V , but for the higher 2/3 of the natural range of v , they accessed as much or more tissue than our best model dendrites to date. This suggested that F is important for most dendrites, and that dendrites of different volumes v should be considered separately. For such an optimum function there should only be tendency toward optimum structures; lesser ones should also occur with a frequency that decreases with F . Thus the distributions of values of features of natural dendrites should resemble plots of F versus that value in optimized dendrites. For natural dendrites, a measure b of daughter to parent branch diameter at branch points is distributed around the value 1. For model dendrites F fell off for small b because V was too small, and for large b because v was too large. The peak value for dendrites varied with v but ranged around 1 in a distribution comparable to the natural one. We can now compare the distributions of F at various v for asymmetry of daughter diameters, sparsity of branching, stem diameter, and other features, to the corresponding natural frequency distributions.

With Dr T.G. Smith we have adapted measurements of a distribution of fractal dimensions ("multifractals") as a mathematical tool to express salient morphological features of cultured neurons and glia.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01686-27 LNLC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Motor Control Systems in the Spinal Cord

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and Institute affiliation)

PI:	R.E. Burke, M.D.	Chief	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLC, NINDS
	G.M. Dold	Engineering Tech.	LNLC, NINDS
	Ely Simon, M.D.	Staff Fellow	LNLC, NINDS
	Alexander Degtyarenko,		
	Ph.D., D. Sc.	Visiting Associate	LNLC, NINDS
	T. Norden-Kritchmar	Computer programmer	LNLC, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.6

PROFESSIONAL:

2.5

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ Human tissues ☒ Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is designed to provide information about the organization of neuronal systems in the mammalian spinal cord that are involved in motor control, as studied in adult cats in vivo and in the isolated brain stem and spinal cord of neonatal rats or mice studied in vitro. Current interest centers on the organization of excitatory last-order interneurons in reflex pathways within the spinal segment, control of information flow in them exerted by other inputs from primary afferent and supraspinal descending systems, and their interaction with the spinal mechanisms that generate rhythmic motoneuron output patterns underlying locomotion. In the cat, we are particularly concerned with interneurons that transmit short-latency excitation from low-threshold skin afferents and from reticulospinal and vestibulospinal systems. All of these systems produce minimally disynaptic excitation in some species of lumbosacral motoneurons and all are powerfully modulated by the spinal central pattern generator (CPG) for locomotion during fictive stepping in decerebrate animals. Differential patterns of CPG modulation are being used to identify separate systems of segmental interneurons, each with highly specific patterns of primary afferent and descending convergence, that are present in the mammalian spinal cord. Work on the in vitro preparation of neonatal rodent spinal cord, with or without the brain stem, is designed in part to develop preparations that can be used to investigate functionally defined reflex pathways, in order to compliment work in the cat. This requires functional isolation of identified peripheral nerves and intracellular recording from select populations of motoneurons and interneurons. We are also investigating a neurological mutant mouse, oscillator (osc), which has a defect in the $\alpha 1$ subunit of the glycine receptor. Attempts are being made to quantitate glycine receptor function at various postnatal ages, using recurrent inhibition. Unfortunately, glycinergic recurrent inhibition in the in vitro preparation is usually superimposed on a glutamatergic synaptic excitation of unknown origin, making it difficult to study glycine responses in isolation. Pharmacological dissection of the responses is being used to deal with this problem.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01687-27 LNLC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Techniques for Making Connections with the Nervous and Musculoskeletal Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and Institute affiliation)

PI:	M.J. Bak	Electronics Engineer	LNLC, NINDS
Others:	R.E. Burke, M.D.	Chief	LNLC, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	M.J. O'Donovan, M.B., Ch.B.	Section Chief	LNLC, NINDS
	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS

COOPERATING UNITS (if any)

Instrumentation and Computer Section, BNP, DIR, NINDS (William Hollsinger)

LAB/BRANCH

Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.2

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ Human tissues ☒ Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project is intended to develop techniques and instrumentation for the acquisition and processing of neuroelectric signals from the central and peripheral nervous systems in acute and chronic neurophysiological preparations. Because of this Laboratory's continuing interest in sensorimotor neural activity during unrestrained movements, the project also includes development and fabrication of chronically implantable microelectrodes, mechanical transducers, catheters, and connectors.

Due to the Laboratory's on going interests in doing research on isolated preparations such as the spinal cord of chicken embryos, a significant amount of work has been devoted to improving techniques associated with electrical recording, stimulation, and real-time fluorescence microscopy in these preparations.

Several projects which have been associated with the visual prosthesis feasibility studies, normally reported on under this project number, are now being reported under project, Z01 NS 02857-02 LNLC. These projects are referenced as such throughout this report. There are also several other new projects which would normally be listed under this project number but are now listed under the recent visual prosthesis project number.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01688-27 LNLC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cortical Mechanisms of Voluntary Motor Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and Institute affiliation)

PI:	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLC, NINDS
	D. Cole	Biologist	LNLC, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	Z. Lee	Summer Intern	University S. CA
	W.J. Heetderks, M.D., Ph.D.	Health Scientist Administrator	DFN, NINDS

COOPERATING UNITS (if any)

Fundamental Neuroscience Program, NINDS (W.J. Heetderks); University of Michigan (K. Wise)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

0.4

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ Human tissues ☒ Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The discovery that activated iridium recording sites have impedance characteristics that are a function of the applied DC bias has led to the investigation of the effects of DC bias on the impedance properties of metals that are commonly used for neuronal recording. Each metal has its own preferred operating potential. An understanding of the basic properties of the microelectrodes should provide improved recording characteristics. Three dimension multicontact passive semiconductor microelectrodes, supplied by the University of Michigan, have been implanted in the arm area of the motor cortex of a primate that was trained to do a number of different wrist movement tasks. Unfortunately, different techniques that were employed to protect the silicon ribbon cable from stresses produced by closing the dura over the microelectrode arrays have all failed. A more robust lead design is required to allow closure of the dura over the microelectrodes.

Two dimension multicontact passive semiconductor microelectrodes, implanted in the supplementary motor cortex of a primate were found to migrate through the cortex, even after months of implantation. Techniques are under investigation that may help to stabilize the location of the microelectrodes for long-term recording.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS02871-04 LN
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Postsynaptic Densities: Mechanisms for Structural Modification		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Ayse Dosemeci, Ph.D.	Visiting Associate LN, NINDS
Others:	Thomas S. Reese, M. D.	Chief LN, NINDS
	Katsuyuki Miyaguchi, Ph.D.	Visiting Associate LN, NINDS
COOPERATING UNITS (if any) Howard Jaffe, Ph.D., Protein/Peptide Facility, LNC, NINDS.		
LAB/BRANCH Laboratory of Neurobiology, BNP, DIR, NINDS		
SECTION Section on Structural Cell Biology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	2.15	PROFESSIONAL: 1.15 OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Postsynaptic densities</u> (PSDs) organize various elements of the postsynaptic response and are likely to play a role in the modification of synaptic efficacy. <u>Calcium</u>-induced changes in PSDs may be involved in the induction and maintenance of activity-dependent synaptic modification. By combined biomedical morphological methods, the project seeks to determine the molecular architecture of the PSD and to identify possible mechanisms for modification by elevated postsynaptic levels of calcium. A major focus has been on CaM kinase II, the most abundant protein in the PSD preparation and a calcium-regulated enzyme implicated in LTP and memory. Previous studies using isolated PSDs indicated that the PSD-associated CaM kinase pool is fully active and has unique properties. In collaboration with Dr. Jaffe, studies to identify autophosphorylation sites of the enzyme are continuing. In addition to Thr 253, described previously, a CnBr cleavage product containing at least three additional potential phosphorylation sites has been identified. Based on the property of CaM kinase II to be phosphorylated at distinct sites in the presence and absence of calcium, a model for its regulation has been devised. A computer simulation of the model (in collaboration with Dr. Albers, LNC) suggests that the autophosphorylation state of an array of CaM kinase II molecules can reflect the frequency of the calcium signal. An important implication of the model is that it provides a potential mechanism for decoding the temporal pattern of synaptic activity. Present studies using subcellular fractions are aimed at testing various implications of the above model. Organotypic hippocampal cultures, developed by Dr. Miyaguchi, are now available to study the regulation of CaM kinase II in intact tissue. Since ischemic has been reported to cause translocation of cytosolic CaM kinase II to the PSDs, we are investigating biomedical and morphological changes associated with ischemic insult to hippocampal cultures. Preliminary results indicate a selective proteolysis following 10 min under ischemic conditions. Future PSD-associated CaM kinase II following specific treatments and to correlate the autophosphorylation state with structural modification. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02873-04 LN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunocytochemistry of Neuronal Cytoplasm

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jorge E. Moreira, Ph.D.	Visiting Scientist	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Sven Beushausen, Ph.D.	Visiting Associate	LN, NINDS

COOPERATING UNITS (if any)

R.Llinas, P.M. Reuss, Dep Physiol and Biophys, NYU Med Cent, NY; LN, Sect Struct Cell Biol, NINDS, NIH, Bethesda, MD; Marine Biological Laboratory, Woods Hole, MA

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, Bethesda, Maryland

TOTAL STAFF YEARS:	0.85	PROFESSIONAL:	0.85	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antibodies to defined domains of the light and heavy chains of the motor protein kinesin (from squid axon) have been used for immunolabeling of freeze substituted squid axoplasm. It was necessary to develop and apply cryogenic methods to prevent displacement of soluble kinesin during tissue processing. We found that kinesins are widely distributed in the cytoplasm but several-fold concentrated around cytoplasmic organelles. The higher concentration is on vesicles (69.6-fold), but increase in gold particles over the cytoplasmic level was also seen around ER cisternae (29.5-fold), microtubules (15.2-fold), and mitochondria (6.2-fold). New experiments using affinity purified polyclonal antibodies against a defined protein fragment of the functional head of the kinesin heavy chain confirmed the previous kinesin location. Western blots performed with the same antibodies, using pure squid kinesin, whole axoplasm, and pellet and supernatant of the centrifuged axoplasm, confirmed the results showing the 116 Kd kinesin band on all the samples. Sections incubated with a polyclonal antibody against squid neurofilament, failed, as expected, to show a selective distribution around vesicles. This work is now complete and ready to publish. It shows that a third of the kinesin in the axon is on organelles while the rest is free in the axoplasm or associated with microtubules. Since the antibody was to the conserved head domain this overall distribution should include several kinesin isoforms.



NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02834-05 LN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of the Excitation-Contraction Coupling Apparatus in Muscle

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Bernhard E. Flucher, Ph.D.	Visiting Associate	LN, NINDS
Others:	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist	LN, NINDS

COOPERATING UNITS (if any)

M.P. Daniels, LBG, NHLBI, NIH, Bethesda, MD.; J.A. Powell, Smith, Northampton, MA.; C. Franzini-Armstrong, Penn. Philadelphia, PA.; K. Beam, Colorado State, Fort Collins, CO.

LAB/BRANCH

Laboratory of Neurobiology, DIR, NINDS

SECTION

Section on Analytical Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892. Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL STAFF YEARS:	0.5	PROFESSIONAL:	0.4	OTHER:	0.1
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to determine the molecular mechanisms involved in the assembly of the triad junction between T-tubules and sarcoplasmic reticulum (SR) during the development of excitation-contraction (E-C) coupling in skeletal muscle. Immunofluorescence studies of the distribution of the skeletal muscle dihydropyridine receptor (DHPR) (the voltage sensor in E-C coupling), the ryanodine receptor (RyR) (the calcium release channel of the sarcoplasmic reticulum) and triadin — both in developing normal muscle and dysgenic (mdg) myotubes in culture — showed that protein-protein interactions mediated by the DHPR plays a role in the normal organization of the triad. Recordings of cytoplasmic free calcium with fluorescent indicators revealed that only action-potential-induced calcium transients are eliminated in the dysgenic mutant. Calcium-induced calcium release events were essentially unaltered in the DHPR null mutant, demonstrating that these events reflect a fundamental and independent function of the RyR. The last studies of this project now show that both of these membrane channels are expressed early in development and appear colocalized in clusters in T-tubules and SR, respectively. Interactions between dihydropyridine and ryanodine receptors are likely to be involved in the clustering process, which presumably represents an early step in triad formation. Parallel investigation of the molecular assembly, the ultrastructure and the developing function of the E-C coupling apparatus during myogenesis indicate that the molecular specialization of junctional T-tubules and SR occurs concomitantly with the initial formation of junctions. Whereas these early junctions exist in a variety of configurations, they are capable of sustaining action potential-induced calcium transients. The maturation of E-C coupling properties can be accounted for by a dramatic increase in junction density that occurs when the junctions associate with the myofibrils.

Due to the departure of the Principal Investigator, this will be the final year of this project.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02835-05 LN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Subcellular Organization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Evelyn Ralston, Ph.D.	Special Expert	LN, NINDS
Others:	Stefanie Kaech, Ph.D.	Visiting Fellow	LN, NINDS
	Michael Cariola	Biologist	
	Ylva Hellsten, Ph.D.	Special Volunteer	
	Christine Winters	Chemist	
	Thorkil Plough, M.D.	Special Volunteer	EDMNS, NIDDK

COOPERATING UNITS (if any)

Jill Horowitz, Division of Hematologic Products, Center for Biologics Evaluation and Regulation, FDA

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.21

PROFESSIONAL:

1.06

OTHER:

0.15

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand how mRNAs, proteins, and subcellular organelles are distributed and organized in muscle and nerve cells. In multinucleated muscle cells, the nonuniform distribution of specific mRNAs and proteins contributes to the formation of the neuromuscular junction. In neurons, differential distribution of specific mRNAs may play a role into the establishment and maintenance of axonal-dendritic polarity. We are trying to understand how mRNA stability and mRNA translation influence mRNA localization. To that effect, we are localizing, by *in situ* hybridization, both endogenous mRNAs and foreign mRNAs from transfected genes, in the mouse muscle cell line C2, and in primary cultures of rat hippocampal neurons. In the past year we have started to consider the role of translation in mRNA localization in neurons, taking as model the mRNA for ferritin, whose translation is regulated by the level of iron in the milieu. After specific inhibition of ferritin, mRNA translation is by a decrease in free iron, ferritin mRNA is found in both cell body. These results suggest that translation of ferritin mRNA is involved in its perinuclear localization. In the future we will continue to investigate the role of this parameter.

We are continuing to investigate the mechanism of vesicle and protein traffic in muscle by studying the localization of the glucose transporter GLUT4 and its translocation to the plasma membrane following stimulation by insulin or exercise. A detailed study of GLUT4 localization in C2 myotubes has been carried out. GLUT4 and several markers of subcellular organelles were localized by single and double immunofluorescence in control myotubes and in myotubes treated with the fungal metabolite Brefeldin A. The results suggest that GLUT4 is stored in vesicles that are specific and distinct from those that carry other markers of the endosomal-lysosomal pathway. These results are significant because specificity of the GLUT4 storage compartment has long been a subject of debate.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS 02836-05 LN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural and Elemental Analysis of Macromolecular Assemblies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist	LN, NINDS

COOPERATING UNITS (if any)

R.D. Leapman, BEIP, NCCR, NIH, Bethesda, MD. J.A. Hunt, Gatan, Inc., Pleasanton, CA

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Analytical Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892; Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL STAFF YEARS:	0.80	PROFESSIONAL:	0.5	OTHER:	0.3
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to characterize *in vitro* the shape, molecular weight distribution and elemental composition of specific individual macromolecules and macromolecular assemblies, with emphasis on components of the cytoskeleton. This project depends on a unique instrument -- a field-emission scanning transmission electron microscope (STEM) -- equipped with dark-field detectors (for mapping the molecular weight distribution of molecules at a spatial resolution of 2 nm) and a parallel electron energy loss (EELS) spectrometer (for detecting physiologically relevant differences in phosphorylation states at a resolution of 10-20 nm). Applying this method to rapidly-frozen, freeze-dried neurofilaments isolated from the squid giant axon, we have been able to derive a novel structural model for the arrangement of heavy chains (highly phosphorylated) and light chains (weakly phosphorylated) in native axonal neuro-filaments. The STEM measurements give a neurofilament mass-per-length of 22.7 ± 0.8 kDa/nm which implies only eight coiled-coil dimers per cross-section. The EELS results indicate four heavy chains per cross section, each containing approximately 50 to 60 phosphates; this is essentially the maximum predicted by the amino acid sequence. We have also begun to exploit an energy-filtering electron microscope (EFTEM) as a complementary approach for the analysis of cellular phosphorus. EFTEM has been previously used for mapping of phosphorus distributions in molecular assemblies, but at high electron dose, which leads to specimen damage. Recent experiments now show that it is possible to obtain quantitative, element-specific images of directly frozen thin films of biological specimens at low dose and therefore potentially at high resolution. Thus, we have obtained phosphorus-specific, low-dose images of herpes simplex virus particles with and without their DNA cores. The difference between these images reveals a statistically significant quantitative map of the distribution of the DNA within the virus. These results suggest that it may be possible to carry out such experiments even in fully hydrated specimens.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS02842-05 LN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Neural Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sven A. Beushausen, Ph.D.	Visiting Associate	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Jim Willard	IRTA	
	Stephanie Kaech	Visiting Fellow	LN, NINDS
	Harish Pant, Ph. D.	Research Chemist	LNC, NINDS
	Ellen Meier, Ph.D.	Senior Staff Fellow	LVMP, NINDS
	Heinz Arnheiter, M.D.	Visiting Scientist	LVMP, NINDS

COOPERATING UNITS (if any)

Professor K.R. Weiss, Ph.D. Mt. Sinai School of Medicine, N.Y.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892.

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

2.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The following summary describes three projects examining the roles of kinesin light chains (KLC), nsec-1 and acidic calponin (AC) play in neural function. The fourth examines the downstream targets of signal transduction enzymes induced at the neuromuscular junction by the myomodulin and buccalin peptide families from the sea hare *Aplysia californica*. KLCs and nsec-1 are proteins that effect synaptic vesicle (SV) transport. We have identified at least nine isoforms of KLC from squid optic lobe and are currently focussing on determining the function of the C-terminal tail domains. We are also pursuing this question *in vivo* by genetic knockout analysis using the technique of homologous recombination. We have previously identified nsec-1 as a regulator of neuronal Cdk5 kinase activity towards neurofilament proteins. Several questions remain regarding how mutation of nsec-1 affects SV trafficking. We are currently endeavoring to identify the remaining components of the nsec-1/Cdk5 complex and determine their respective functions as they relate to Cdk5 activity. AC is a nonsmooth muscle isoform of basic calponin that we have shown localizes to the developing growth cones of neurons. AC levels diminish as neurons differentiate and a number of regulatory domains in the unique C-terminal tail domain including a PEST domain, an isoprenylation site, a P-loop element and a tyrosine phosphorylation site all suggest mechanisms for regulating protein second messenger-induced translocation and protein turnover. Current research is directed toward addressing how these domains relate to protein function. We have also participated in identifying twitchin, a 750 kDa muscle protein, as a primary target of PK-A phosphorylation following ARC muscle stimulation with the neuro peptide myomodulin in *Aplysia californica*. Further work is directed towards elucidating receptors and other downstream targets of the signal transduction cascades in response to stimulation by the neuropeptides buccalin and myomodulin.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS-02869-09 LN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Influence of Leukocytes on Neural Growth

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Lisa Chang, B.S.	Biologist	LN, NINDS
Others:	Milton Brightman, Ph.D.	Section Chief	LN, NINDS

COOPERATING UNITS (if any)

Wu Ma, Ph.D., Staff Fellow, LNP, NINDS

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Brain Structural Plasticity,

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

0.5

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal remains to determine the extent to which axons of a damaged spinal cord can be induced to regenerate. The spinal cord of adult rats is crushed epidurally at T-9 in rats. Axons have been identified by immunostaining for neurofilaments. However, as in all other reported cord traumas, regardless of the method of injury used, assessment of axon regeneration has been thwarted by the inability to distinguish intact, preexisting axons from newly regenerating ones. The current collaborative finding may resolve this dilemma. E10, the mRNA expressing the embryonic form of glutamic acid decarboxylase (GAD in the cord, disappears by adult life. It is now found that E-10 is reexpressed in regenerating spinal cord. The expectation is that antibodies to the embryonic form of GAD will enable its detection in axons and thus permit distinction, for the first time, between regenerating fibers and preexisting ones. In a new series, cord-injured rats are given only the free radical suppressor, deprenyl, and permitted to survive for 1 d to 5 w after the injury. Immunohistochemical identification of the predominant cell types at different stages of wound repair is being made so that a sequence of administering cytokines and growth factors, secreted by leukocytes, can be established.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02610-12 LN

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Elemental and Structural Organization of Neurons and Glia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Natalia Pivovarova, Ph.D.	Visiting Fellow	LN, NINDS
	Diane D. Murphy, Ph.D.	IRTA Fellow	LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist	LN, NINDS

COOPERATING UNITS (if any)

R. Leapman, BEIP, NCCR, NIH, M. Segal, LAS, NINDS, NIH, D. Landis, Case-West. Res Univ, Cleveland, OH; B. Trapp, Clev. Clin, OH; R. Buchanan, Ark. State Univ, State College, AK; J. Connor, Roche Inst, Nutley, NJ

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Analytical Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892; Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL STAFF YEARS:

2.3

PROFESSIONAL:

1.7

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This two-part project studies the organization and function of specialized membranes in neurons and glia. The first part aims to characterize calcium regulation during synaptic activity in the dendrites and dendritic spines of cerebellar Purkinje cells and CA3 hippocampal pyramidal cells. Previous electrophysiological and structural work had established that a new type of organotypic culture of hippocampus was an excellent preparation for studying free calcium transients in living slices, as well as for analyzing the cellular distribution of total calcium in directly frozen cultures by means of analytical electron microscopy. Further experiments have now revealed a dramatic increase in the calcium content of specific endoplasmic reticulum-like organelles within the dendrites of CA3 neurons under conditions of synaptic stimulation which produce a response similar to long-term potentiation (LTP). This provides the first direct evidence for and identification of calcium-sequestering organelles which are specifically involved in potentiating neural-inducing activity. Parallel experiments on cultures of isolated pyramidal cells have revealed a two-fold increase in the density of dendritic spines on neurons treated with estradiol during development of the dendritic arbor. This preparation appears promising for studying the development of the calcium-regulating apparatus in conjunction with hormonal effects on the development of spines and synapses. In Part Two, immunocytochemistry and confocal light microscopy have been used to study the expression, transport and assembly of myelin. Prior work had shown that myelinating Schwann cells have a characteristic organization of microtubules which is essential for the sorting, transport and targeting of myelin-specific proteins. We have now used microtubules assembly/disassembly experiments to show that an axon-specific signal mediates the appropriate organization of Schwann cell microtubules, i.e., the dispersion of microtubule minus ends and the induction of multiple microtubule organizing centers in perinuclear cytoplasm. This signal is absent during Wallerian degeneration, which therefore results in formation of disruptive microtubule organizing centers within the myelin internode.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02551-14 LN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Cytoplasm Motors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS
Others:	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Mark Terasaki, Ph.D.	Senior Staff Fellow	LN, NINDS
	Shahid Khan, Ph.D.	Guest Researcher	LN, NINDS
	Alexandra Schmidek	Summer Student	LN, NINDS

COOPERATING UNITS (if any)

E. Bearer, Dep Pathol, Brown Univ; R.D. Leapman, BEIP, NCCR, NIH, Bethesda, MD; B.J. Schnapp, Dep. Cell. Mol. Physiol., Harvard Med. Sch., Boston, MA. Lab Neurobiol, DIR, NINDS; Marine Biol Lab, Woods Hole, MA

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	0.75	PROFESSIONAL:	0.75	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The goal of this project is to understand the distribution and functions of cytoplasmic motors in the axon of neurons. This information is intended to lead to an understanding, at the molecular level, of fast and slow axonal transport as well as the cytoplasmic organization in the axon. This is the central project in the Section, and has been very productive this year, providing a plethora of new findings. A novel subclass of myosin II-like motors on the surfaces of squid axonal organelles has been discovered and this myosin as well as its calmodulin-like light chain can now be purified in quantity from brain, and is being characterized. Immunolabeling shows that this myosin adheres strongly to the surfaces of axonal organelles. The organization of the actin substrates for these motors in the axon has showed that actin filaments intertwine with the microtubule bundles. An assay that may be useful for approaching the difficult problem of defining the mechanism of slow transport has also been introduced. Macromolecular assemblies injected into the squid giant axon are move in the anterograde direction at rates up to 0.5 Mm/sec. Of particular interest is that short actin filaments also move anterogradely and that all movements appear to be along some type of intracellular tract. This *in vitro* assay should make it possible to define the motors for such movements. The bacterial flagellar motor in *E. coli* has been studied as another example of a motor system than can switch direction of translocation. A new cytoplasmic component of the flagellar motor thought to be involved in directional switching has now been isolated and purified, and we are making progress in devising a structural model of how the proteins in this component of the motor are organized.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02144-21 LN

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Blood-Brain Barrier

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Mehmet Kaya, Ph.D.	Visiting Fellow	LN, NINDS
Others:	Elena Sanovich, Ph.D.	Special Volunteer	LN, NINDS
	Lisa Chang, B.S.	Biologist	LN, NINDS
	Milton Brightman, Ph.D.	Section Chief	LN, NINDS

COOPERATING UNITS (if any)

Raymond Batus, Ph.D. Senior Staff Research Scientist, Alkermes, Inc. Cambridge, MA

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.9

PROFESSIONAL:

2.5

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) Blood-brain barrier vessels (BV) can be converted to permeable, fenestrated vessels (FV) *in vivo* by chemical means. Phorbol ester retinoic acid infused into rats' cerebral cortex convert about 30% of vessels to FV by 28 days. Accordingly, blood-borne agents may be brought into the brain continuously for indefinite periods. (2) There is a short fetal period when skeletal muscle grafted to mature choroid plexus is supplied by FV as well as the expected continuous muscle type (CV). It is hypothesized that a conversion factor, changing CV to FV, is secreted by E14 but not E16 muscle. The grafts' FV are derived from choroid plexus, whose vessels became labeled with ^3H -thymidine; but no graft vessels were tagged, maybe because tracer had not been injected more often. Vascular endothelial growth factor is not the converter because neither the factor itself nor its mRNA, were in the grafts. (3) The blood-brain barrier of about 20% of normal mouse brain vessels was opened to La^{3+} with RMP-7, an analog of bradykinin. RMP-7 acts by, presumably, binding to its endothelial B-2 receptor with the result that endothelial tight junctions become permeable, allowing La^{3+} to pass through to them into the brain.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02086-22 LN
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regeneration Specificity in Transplanted Neural Tissue		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I. Jung-Hwa Tao-Cheng, Ph.D. Unit Chief EM Facility, LN, NINDS Others: Milton Brightman, Ph.D. Section Chief LN, NINDS		
COOPERATING UNITS (if any) David Simpson, Ph.D., DRG, NINDS, Joseph Bressler, Ph.D., Kennedy Institute, Baltimore, MD		
LAB/BRANCH Laboratory of Neurobiology, BNP, DIR, NINDS		
SECTION Section on Brain Structural Plasticity		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"><div style="width: 30%;"><input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews</div><div style="width: 30%;"><input type="checkbox"/> (b) Human tissues</div><div style="width: 30%;"><input checked="" type="checkbox"/> (c) Neither</div></div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project has been completed and is terminated.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01881-25 LN
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structural Basis of Synaptic Transmission		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Katsuyuki Miyaguchi, M.D. Visiting Associate LN, NINDS Others: Jorge E. Moreira, Ph.D. Visiting Scientist LN, NINDS		
COOPERATING UNITS (if any) R.Llinas, P.M. Reuss, Dep Physiol and Biophys, NYU Med Cent NY; Sect StructCell Biol, NINDS, NIH; Marine Biol Lab, Woods Hole, MA		
LAB/BRANCH Laboratory of Neurobiology		
SECTION		
INSTITUTE AND LOCATION NINDS, Bethesda, Maryland		
TOTAL STAFF YEARS: 0.65	PROFESSIONAL: 0.65	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project deploys a range of structural techniques to examine normal <u>synaptic plasticity</u> and development. These approaches have in common their dependence on <u>rapid-freezing</u> and direct visualization of living brain by light microscopic techniques. This project has been engaged in exploring various live brain preparations suitable for these purposes. Success has been achieved with a new approach to culturing <u>hippocampal slices</u>. Their typical laminar organization and most of their thickness can be maintained for up to 12 weeks by culturing them at the interface between air and culture medium. Structural and electrophysiological studies of the slices from different ages have been investigated. Within 10 days in culture, CA1 dendrites had large filopodia-like structures containing a number of sERs and almost no spines. Extracellular field EPSP's (fEPSP) amplitude was 3.5 ± 1.1 mV and tetanic stimulation (100Hz, 1sec) caused no LTP. At 3 weeks, spines were present in large heterogeneity, fEPSP amplitude was 5.6 ± 0.9 mV, and tetanic stimulation caused LTP in 63% of the slices. At 5 weeks, spine density was higher, but no significant increase in the fEPSP amplitude or ability to produce LTP was observed. The ability to express LTP appeared to be correlated with the appearance of the spines. Ultrastructural and immunocytochemical studies comparing immature and matured spines, and the effect of various kinds of chemicals which control synaptic activities will be investigated. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01442-29 LN									
PERIOD COVERED October 1, 1994 to September 30, 1995											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Permeability of Cellular Layers in the Vertebrate Nervous System											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"><tr><td style="width: 33%;">PI:</td><td style="width: 33%;">Thomas S. Reese, M.D.</td><td style="width: 33%;">Chief</td><td style="width: 33%;">LN, NINDS</td></tr><tr><td>Others:</td><td>Bechara Kachar, M.D.</td><td>Visiting Scientist</td><td>LNO, NIDCD</td></tr></table>			PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS	Others:	Bechara Kachar, M.D.	Visiting Scientist	LNO, NIDCD	
PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS								
Others:	Bechara Kachar, M.D.	Visiting Scientist	LNO, NIDCD								
COOPERATING UNITS (if any) Mar Biol Lab, Woods Hole, MA; Woo Kuen-Lo, Ph.D., Dep Anat, Morehouse Med Sch, Atlanta, GA; LN, Sect Struct Cell Biol NINDS, NIH											
LAB/BRANCH Laboratory of Neurobiology											
SECTION Section on Structural Cell Biology											
INSTITUTE AND LOCATION NINDS, Bethesda, Maryland											
TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"><tr><td><input type="checkbox"/> (a) Human subjects</td><td><input type="checkbox"/> (b) Human tissues</td><td><input checked="" type="checkbox"/> (c) Neither</td></tr><tr><td><input type="checkbox"/> (a1) Minors</td><td></td><td></td></tr><tr><td><input type="checkbox"/> (a2) Interviews</td><td></td><td></td></tr></table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is now in abeyance.											

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02913-01 LNC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

LNC-NINDS Protein/ Peptide Sequencing Facility

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Howard Jaffe, Ph.D. Special Expert LNC, NINDS

COOPERATING UNITS (if any)

S Beushausen, NB, NINDS, NIH; A Dosemeci, LN, NIH, NINDS; J Hallenbeck, DIR, SB, NINDS, NIH; R McCarron, SB, NIH, NINDS

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The LNC-NINDS Protein/Peptide Sequencing Facility has continued to provide NINDS investigators with theoretical and technical expertise for the separation, purification, and amino acid sequencing of peptides and proteins. During the past year, 9 service mode amino acid sequencing projects have been completed, on peptides, fusion or endogenous proteins, or enzymatic or chemical fragments of proteins. Since its opening in 1922, 48 cooperative projects have been undertaken. Of these projects, which represent the main activity of the Facility, 20 were completed and 28 continued during the year. Highlights of three cooperative projects are described: (1) A 67 kDa cdc2-like kinase activator from rat spinal cord was isolated and purified. The protein was subjected to enzymatic degradation and the resulting peptide fragments purified by narrowbore RP-HPLC and sequenced. Sequences representing ca. 17% of the molecule were used to design the cDNA probes. Using oligonucleotide primers deduced from these amino acid sequences, full-length cDNAs have been obtained from rat brain libraries in conjunction with the techniques of RT/PCR and cDNA library screening. (2) Several proteins that may be involved in hibernation in the thirteen-lined ground squirrel (TLS) have been identified. A potential hibernation protein was submitted to the Facility as a 2D-PAGE PVDF blot and subjected to *in situ* proteolytic digestion. The protein was identified on the basis of a internal sequence as phosphoglycerase mutase. Plasma factors related to hibernation were purified by a combination of HP-SEC and RP-HPLC. Two proteins (30-70 kDa) elevated in the nonhibernating TLS plasma and essentially absent in the hibernating plasma, were identified by combination of N-terminal and internal amino acid sequencing as the previously reported HP-55 alpha-1 -antitrypsin and a previously unreported alpha-1-antitrypsin. The N-terminus of the unreported alpha-1-antitrypsin appears to significantly differ from others in the Swiss protein database, but is clearly placed in this family in the basis of its internal amino acid sequences. Two additional proteins were identified from lower molecular weight fractions. The first, is the known protein, apolipoprotein, and the second (elevated in the hibernating plasma), is a previously unreported protein, which upon proteolytic digestion yielded sequences, unmatched in the Swiss protein database. (3) In analogy to soluble CaM kinase II, postsynaptic density-associated CaM kinase II undergoes autophosphorylation in the presence of calcium/calmodulin. Thr (253) was identified as the major autophosphorylation site by sequencing of the radioactive peptides resulting from proteolytic cleavage of radiolabeled postsynaptic density-associated CaM kinase II. Significantly, phosphorylation at this site under similar conditions has not been reported in the soluble kinase.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02874-03LNC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Studies of GABA_A Receptor Expression in the Developing CNS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Lawrence C. Mahan, Ph.D.	Research Cell Biologist	LNC, NINDS
Others:	Peng-Xin Lin, M.D.	Visiting Associate	LNC, NINDS
	Peter M. Geiger	Biologist	LNC, NINDS
	Kiyoshi Kusano, Ph.D.	Visiting Scientist	LNC, NINDS

COOPERATING UNITS (if any)

AThierry, LTCB, NCI, NIH; C Felder, LCB, NIMH, NIH

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Molecular Neurosciences

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.05

PROFESSIONAL:

2.2

OTHER:

0.85

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have used pluripotent P19 embryonic carcinoma stem cells as an *in-vitro* system to study the early neuronal expression and function of GABA and subunit mRNAs of the GABA(A) receptor. These cells are differentiated with retinoic acid (RA) into GABAergic neurons and glia and maintained in culture for 30-40 days *in vitro*; DIV. We have shown that the differentiation of P19 cells into neurons occurs in a synchronous manner both by the immunocytochemical detection of sequential expression of stage-specific markers and an analysis of neuronal mitosis using BrdU pulse-labeling. The acquisition of functional responses to GABA coincided with the differentiation of P19 cells from neuroprogenitors into post-mitotic "embryonic-like" neurons. Moreover, electrophysiological analyses revealed that GABA, acts in an autocrine manner to produce excitatory membrane responses as early as 3-4 DIV post-RA. These responses mature into repetitive, all-or-none action potentials by 8-10 DIV post-RA. Using single cell Fura-2 image analysis, we demonstrated that GABA-elicited action potentials result in neuron-specific increases in free intracellular calcium, in contrast to the hyperpolarizing action of GABA in adult neurons. We then used RT-PCR to determine the temporal (5-30 DIV) expression of 13 subunit mRNAs of the GABA(A) receptor in P19 cells during *in vitro* differentiation. This *in vitro* pattern of expression of subunit mRNAs ($\alpha 3/5 > \alpha 1/2/4 > \alpha 6$; $\beta 3 > \beta 2 > \beta 1$; $\gamma 1 > \gamma 2/\gamma 3$) closely resembled the pattern of expression revealed by ISHH studies *in vivo* in the mammalian CNS shortly after neurogenesis and prior to mature synaptogenesis and histogenesis. This model system will allow us to examine to what degree the expression of subunits of the GABA(A) receptor in developing neurons is determined by an intrinsic program of gene expression or directed by local environmental cues such as growth and differentiation factors or GABA itself. We are currently developing single-cell mRNA/PCR detection techniques to examine the combinational subunit heterogeneity within P19 neurons (and possible glial progenitors) and to correlate subunit composition to functional properties of the receptor. In addition, we are utilizing molecular biological approaches to apply antisense oligonucleotides, and construct mammalian expression vectors capable of sustained cDNA expression in precursor and/or terminally differentiated neurons, to knockout or alter GABA(A) receptor function in these cells. These approaches may provide both an understanding and permit manipulation of the earliest expression of subunit genes of the GABA(A) receptor in order to better define a developmental role for GABA and its receptor in the mammalian CNS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02824-05 LNC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development and Regulation of the Luteinizing Hormone Releasing Hormone (LHRH) System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Susan Wray, Ph.D.	Research Cell Biologist	LNC, NINDS
Others:	Sharon Key	Biologist	LNC, NINDS
	Susan Fueshko, Ph.D.	IRTA Fellow	LNC, NINDS
	Jennifer Maurer, Ph.D.	PRAT Fellow	LNC, NINDS
	Peter Geiger	Biologist	LNC, NINDS

COOPERATING UNITS (if any)

B Olson, Neuroend Div, NICHD, NIH; S Radovick, Div. Endocrinol, Child. Hospital, Boston, MA; R Weiner, Reprod Endocrinol Ctr, UCSF, San Francisco, CA; S Ojeda, Neuroscience Div., OR Reg Prim Ctr., Beaverton, OR

LAB/BRANCH

Laboratory of Neurochemistry, BNP, DIR, NINDS

SECTION

Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	3.55	PROFESSIONAL:	2.5	OTHER:	1.05
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Luteinizing hormone releasing hormone (LHRH) neurons are derived from the olfactory placode and migrate into the brain, where they become integral members of the hypothalamic-pituitary-gonadal axis. We study the mechanisms underlying LHRH neuronal migration into the CNS in normal and transgenic animals, as well as in nasal explants. The intrinsic and transsynaptic regulation of LHRH gene expression, peptide synthesis and secretion in postnatal LHRH neurons (in the CNS) vs embryonic LHRH neurons (outside the CNS) is studied using long-term organotypic cultures and nasal explants, respectively.

Working on the hypothesis that LHRH neurons migrate on peripherin positive (P+) olfactory axons via adhesion between cell surface molecules, we are examining specific carbohydrate moieties which might "highlight" these adhesive molecules. Using lectin cytochemistry, we found that: 1) olfactory axons in nasal explants express several sugar moieties; 2) the pattern of carbohydrate expression *in vitro* is similar to that *in vivo*; and 3) *in vitro*, D-N-acetyl-glucosamine oligomers are detected on P+ axons with which LHRH neurons are associated and are localized to LHRH neurons which migrated out of the explant. Using tunicamycin, we found that during an early time window, inhibition of N-glycosylation prevents outgrowth of P+ axons, but does not affect the association of LHRH neurons with these fibers. Using the LHRH promoter fused to a luciferase reporter, we attempted to monitor migrating LHRH neurons *in situ*. However, the luciferase signal was not robust enough to detect. We have now fused the LHRH promoter to the Lac Z reporter and are currently working on optimizing a fluorescent signal using this tag.

Using two different transcription inhibitors, we have broadened our studies on stability and turnover rates of LHRH mRNA. Using 2nd messenger analogs, we are examining (1-24 hr after stimulation) changes in LHRH mRNA to determine a) the 2nd messenger systems active in LHRH cells; and b) an optimal timepoint to monitor changes in LHRH mRNA levels after stimulation with neurotransmitters.

Currently, we are determining: 1) the mechanism by which N-glycosylation "directs" outgrowth of P+ axons; 2) the role of molecules expressing D-N-acetyl-glucosamine oligomers on LHRH neuronal movement and/or olfactory axon outgrowth; 3) whether LHRH neurons in cultures release LHRH in a pulsatile manner; 4) whether tagged-LHRH neurons can be visualized *in situ* to monitor movement in embryonic explants and/or determine the membrane properties of postnatal LHRH neurons in organotypic slices; and 5) the effect of GABA on LHRH gene expression in embryonic and postnatal LHRH neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02828-05 LNC
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Molecular Analysis of Calcium Channel Gene Expression in Mammalian Nervous System		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
P.I.	Hemin Chin, Ph.D.	Research Cell Biologist LNC, NINDS
Others:	Dong-Sun Kim, Ph.D.	Visiting Fellow LNC, NINDS
	Oh-Joo Kwon, M.D., Ph.D.	Visiting Fellow LNC, NINDS
	Kyung Hea Cho-Park, Ph.D.	Special Volunteer LNC, NINDS
	Susan Wray, Ph.D.	Research Cell Biologist LNC, NINDS
	Harold Gainer, Ph.D.	Laboratory Chief LNC, NINDS
	Dan Xi, Ph.D.	IRTA Fellow LNC, NINDS
COOPERATING UNITS <small>(if any)</small> DG Puro, University of Michigan School of Medicine, Ann Arbor, MI; CA Kozak, LMM, NIAID, NIH; E Stanley, SMS, NINDS, NIH		
LAB/BRANCH Laboratory of Neurochemistry, NINDS, BNP, DIR		
SECTION Molecular Neuroscience		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	3.9	PROFESSIONAL: 3.9 OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p> The long-term goals of this project are: 1) to understand the molecular basis for cell type-specific expression of <u>voltage-sensitive calcium channel (VSCC) genes</u>, and 2) to study the roles of VSCCs and <u>synaptic vesicle-associated proteins</u> in the process of <u>secretion</u> at <u>peptidergic nerve terminals</u>. Expression of diverse VSCC subtypes appears to be highly regulated both spatially and temporally during development of the central nervous system. To examine the molecular regulatory mechanisms underlying cell-specific expression of VSCC genes, we have chosen <u>dihydropyridine-insensitive N-type</u> and <u>dihydropyridine-sensitive L-type alpha 1 subunit genes</u>, since expression of <u>N-type channels</u> is restricted to neurons particularly in nerve endings while <u>L-type channels</u> are present in many excitable cell types and also predominantly in neuronal perikarya. Deletion analysis using reporter-alpha 1 subunit fusion gene constructs carried out during last year has identified several positive and negative regulatory elements located at both proximal and distal to the 5' flanking promoter regions of the N-type alpha 1B and L-type. We plan to further characterize the interactions between these regulatory elements and <u>transcription factors</u> that either enhance or repress expression of VSCC alpha 1 subunit genes in cell type- or tissue-specific manner. Biochemical and molecular analyses of interaction of VSCC with the proteins involved in the synaptic vesicle cycle aim to understand the roles of N-type and L-type VSCC in synaptic secretion. We and others have shown that the N-type VSCC, which is involved in fast secretion, interacts with the vesicle docking proteins <u>syntaxin</u>, <u>SNAP-25</u>, <u>Munc-18</u> and the calcium binding protein <u>synaptotagmin</u>. To identify and characterize the putative calcium sensor in "slow" neurosecretion, we have isolated a novel member of synaptotagmin gene family from rat hypothalamus. This synaptotagmin isoform (Syt B/K) is distinct from other synaptotagmins in that its mRNA is highly abundant in the rostral portion of adult rat brain and surprisingly in the kidney. The possible role of Syt B/K in stimulus-secretion coupling and vesicle trafficking will be examined. In addition, we plan to examine expression of VSCC subtypes and synaptic vesicle-associated proteins present in the peptidergic nerve terminals and characterize protein-protein interactions among them. These comparative studies will provide insights into how molecular specificity for <u>stimulus-secretion coupling</u> is determined. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02820-06 LNC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cloning and Functional Analysis of Genes Active in Neurogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Ward F. Odenwald, Ph.D.	Tenure-Track Investigator	LNC, NINDS
Others:	Ravi Kambadur, Ph.D.	Visiting Associate	LNC, NINDS
	Shang Ding Zhang, M.D.	Visiting Associate	LNC, NINDS
	Peter Vos, Ph.D.	IRTA Fellow	LNC, NINDS
	Chad Stivers	Pre IRTA Fellow	LNC, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology (Neurogenetics Unit)

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	3.9	PROFESSIONAL:	3.65	OTHER:	0.25
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this program is to identify and functionally characterize neurogenic genes that are required for CNS development. Given the high degree of conservation in basic developmental mechanisms used by all metazoans, we have focused our efforts on the study of CNS development in the fruit fly (*Drosophila melanogaster*) where the genetic information required for these events is accessible. Using classical genetic, molecular biology and transgenic techniques, we have continued to study the function of genes expressed during neuroblast lineage differentiation. Thus far, our study of castor, a novel Zinc finger gene required for proper CNS neuroblast development, has revealed that it encodes a nuclear located, sequence-specific DNA-binding protein whose expression is restricted to late forming CNS neuronal precursor lineages. We hypothesize that castor protein functions as a transcription factor required for the correct regulation of genes in these lineages. Our recent studies have shown that castor controls cell fate decisions by regulating the expression of known CNS cell fate determinant transcription factors (*pdm-1*, -2 and *drifter*). While castor is required for the silencing of both *pdm-1* and -2 gene expression (early lineage determinants), *drifter* requires castor for its proper expression. A search for additional regulatory targets of castor has identified in vivo DNA-binding sites that are positioned adjacent to genes expressed during CNS development. Recent analysis of one of these targets, the heat-shock gene, hsp-27, has shown that, in the absence of heat induction, castor function is necessary for its proper expression in a subset of late forming neuroblast lineages. We have also continued our characterization of castor cognate genes in other diptera. Information obtained from these comparisons will aid in our identifying mammalian cognates. Our continued analysis of pollux a membrane associated adhesion protein encoding gene has revealed that it contains a highly conserved 74 amino acid protein domain that is found in plant and human proteins. We hypothesize that this adhesion molecule plays a role in both the maintenance of CNS axon fascicles and in proper trachea function. We have also continued our functional analysis of the murine homeobox gene A5. Ectopic expression of A5 in transgenic mice correlates with the apparent repression of a hepatocyte nuclear transcription factor (HNF-3 β) in adult tissues and in skeletal malformations during development.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02757-08LNC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Studies of Peptidergic Neurons and Peptide Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Kiyoshi Kusano, Ph.D.	Visiting Scientist	LNC, NINDS
Others:	Harold Gainer, Ph.D.	Laboratory Chief	LNC, NINDS
	Susan Wray, Ph.D.	Research Cell Biologist	LNC, NINDS
	Shirley House, B.S.	Biologist	LNC, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.05

PROFESSIONAL:

0.9

OTHER:

0.15

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Autoregulatory effects of oxytocin (OT) and vasopressin (VP) on the activities of identified OT and VP neurons were studied in dissociated cell cultures. Our aims were to: 1) establish *in vitro* preparations of dissociated postnatal magnocellular neurons (MCNs) from supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus; 2) identify the OT and VP phenotypes of the electrophysiologically-analyzed MCNs by immunocytochemistry; and 3) study the interactions of OT and VP with amino acid receptors and voltage-gated ion channels. Dissociated SON-MCNs and PVN-MCNs, prepared from PN6-12 rats, were plated on monolayer-cultured brain astrocytes and maintained for up to five weeks. Patch-clamp methods were used for both whole-cell current clamp and voltage-clamp analyses. Patch-pipettes were filled with various modified intracellular solutions containing 5 mM biocytin. The biocytin was used to identify the recorded cells. Neurotransmitters were applied to the cells through small pipettes by pressure pulses. To identify phenotypes of MCNs, antibodies raised to VP (VA4) and OT (PS36) were used. The best result was obtained using a triple fluorescent dye staining; VP-cells with goat anti-rabbit-FITC, OT-cells with goat anti-mouse Texas red, and the biocytin-injected cells using avidin-conjugated cascade blue. Spontaneous activity of intrinsic origin and via synaptic activation were observed in high density, but not in low density MCN cultures. Almost all MCNs were responsive to glutamate and GABA. Glutamate receptor-activated currents were excitatory and GABA receptor-activated currents were inhibitory. Half of the cultured MCNs showed excitatory responses to histamine. Other transmitter candidates (ACh, */*-EP, DA, 5HT, CCK-8, enkephalin, GRP, OT, VP, and somatostatin) were examined using the puffing method, but did not induce membrane current flow in MCNs. However, some of these agents (*/*-EP, CCK-8, OT, VP) did induce small and slow membrane potential changes by bath application. Modulation of the excitatory (KA or NMDA) or inhibitory (GABA) amino acid (10^{-4} M) responses by OT and VP was examined by bath-application of 10^{-7} M OT or VP. Preliminary results suggested that OT and VP feed back on the MCNs produce activation as well as inhibition.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS-02723-09LNC

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Peptides in Adult and Developing Vertebrate Nervous Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Harold Gainer, Ph.D.	Laboratory Chief	LNC, NINDS
Others:	Susan Wray, Ph.D.	Research Cell Biologist	LNC, NINDS
	Shirley House	Biologist	LNC, NINDS
	Diane Witt, Ph.D.	IRTA Fellow	LNC, NINDS
	S-W Jeong, M.D., Ph.D.	Visiting Fellow	LNC, NINDS
	Hemin Chin, Ph.D.	Research Cell Biologist	LNC, NINDS
	Peter Geiger	Biologist	LNC, NINDS

COOPERATING UNITS (if any)

M Castel, Hebrew University, Israel; M Morris, Wake-Forest University, Winston-Salem, NC; H Arnheiter, LDN, NINDS, NIH, J Nagle, DNA Facility, NINDS, NIH

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.2

PROFESSIONAL:

3.3

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to study mechanisms underlying the developmental and homeostatic regulation of the gene expression, posttranslation modification, and secretion of neuropeptides with a focus on the magnocellular oxytocin (OT) and vasopressin (VP) neurons in the hypothalamus. These neurons have historically served as valuable models for cell biological studies of peptidergic neurons, because of the relative ease by which the perikarya, axons, and nerve terminals of these cells *in vivo* can be isolated for anatomic, biochemical, and physiological analysis. Our general approach to experimental perturbation of molecular mechanisms in this system is to harness the regulatory elements in the OT and VP genes in transgenic mice in order to target specific molecules to these cells *in vivo*. The OT and VP peptide genes are good sources for regulatory controls since they are relatively abundantly and specifically expressed in these neurons in the CNS. The major problem has been to identify the critical regulatory elements in these genes which are responsible for the cell-specific expression. Recent transgenic studies have implicated various untranslated nucleotide sequence regions in the OT and VP genes which appear to contain these regulatory elements. Based on these studies, we have hypothesized that the 3.5 kb intergenic regions (between the OT and VP genes) in the mouse contains these cell-specific enhancers. In the past year, we have completely sequenced this intergenic region (IGR) and have found many putative regulatory motifs in this domain. In addition, two constructs containing the upstream regions of either the OT or the VP gene connected to the reporter protein, lacZ, followed by the IGR have been prepared for transgenic mouse experiments now in progress. Given success of our constructs in specific targeting in the OT and/or VP cells, we will replace the lac-Z in the constructs with oncogenes (e.g., SV40 T-antigen) in an effort to generate OT and VP synthesizing cell lines. In the absence of such lines, we have developed several slice-explant systems to study OT and VP gene expression *in vitro*. These range from acutely prepared (<8 hr) slices to long-term cultured (3-4 weeks) slice-explants which remain "organotypic" in topography. The former (acute) systems have been very valuable for our studies on the role of calcium in immediate-early gene expression (e.g., c-fos), while the long-term cultures have been useful models for the study of OT and VP mRNA turnover and regulation in response to neurotransmitter and second message stimulation. In addition to the roller-culture versions of the "organotypic" cultures that we previously used, we have also recently employed "stationary" slice-explant cultures on millipore filters. The latter cultures also thin with time, remain even more "organotypic" structurally, and allow for the long-term survival of both OT and VP magnocellular neurons as well as the suprachiasmatic nucleus. Efforts to transfect cells in these slice-explants with various gene constructs using biolistic (particle mediated transfer of genes) techniques has met with partial success.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02724-09 LNC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Basic Cell Biological Mechanisms Using the Squid Nervous System Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Harish C. Pant, Ph.D.	Research Chemist	LNC, NINDS
Co-PI:	Harold Gainer, Ph.D.	Laboratory Chief	LNC, NINDS
Others:	Shirley B. House, B.S.	Biologist	LNC, NINDS
	Philip Grant, Ph.D.	Special Volunteer	LNC, NINDS
	Hemin Chin, Ph.D.	Research Cell Biologist	LNC, NINDS
	M. Takahashi, M.D., Ph.D.	Visiting Fellow	LNC, NINDS
	Dong-Sun Kim, Ph.D.	Visiting Fellow	LNC, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology/Enzyme Chemistry

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	1.35	PROFESSIONAL:	1.3	OTHER:	0.05
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project contains two specific goals: 1) To study the cell biological mechanisms in axonal phosphorylation/dephosphorylation of neurofilament proteins; and 2) to test the hypothesis that axonal protein synthesis occurs in the squid giant axon. For the latter study, we generated specific antibodies and cDNA probes for squid neurofilament (NF) proteins to study protein synthesis in stellate ganglia (cell bodies) and in axons. Protein biosynthesis/immunoprecipitation experiments confirmed robust biosynthesis of NF proteins in squid stellate ganglia, but we failed to detect any NF protein biosynthesis in the giant axon. This is in the face of our findings of significant NF protein mRNA in the squid axon. In the other study, we have examined the dynamic equilibrium of phosphorylation/dephosphorylation reactions mediated by multimeric complexes of kinases and phosphatases, their regulators and inhibitors. We believe these complexes are compartmentalized in axons and cell bodies and suggest that they play an important role in assembly and transport of cytoskeletal structures and organelles. In the neuronal cell bodies, there is a delicate balance between factors regulating the activity of kinases and phosphatases that modulate the incomplete phosphorylation (e.g., head domains only in NFs) of NFs and tau prior to their assembly and transport into the axon. In axons, NFs are further phosphorylated (in tail domains) and organize into a lattice that stabilizes the tau-bound MT bundles involved in the transport of organelles. To test the above hypothesis, we used P13^{suc1} sepharose-conjugated beads to extract the kinases that phosphorylate neurofilaments in the axoplasm from the squid giant axon. Using Western blots and in vitro kinase assays, we demonstrated the presence of an active cdc2-like kinase and its putative regulators such as cyclin E, P13 homologue and P67 in axoplasm and a P13-axoplasm complex (P13-Ax). Protein kinase A (PKA), casein kinase (CK) I and II were also found in the P13-Ax. Western blot analysis of the P13-Ax also demonstrated several axonal cytoskeletal components; e.g., neurofilaments (NFs; NF 60, 70 and 220), tubulin, actin and microtubule associated proteins. NF 220 and tubulin were phosphorylated by the kinases in the P13-Ax. To determine whether NFs bound directly to the P13 beads, or bound indirectly by association with cdc2 kinase, a washed, axon-derived neurofilament preparation that contained NFs, PKA, CKI and tubulin, but no cdc2-like kinase, yielded no bound proteins after incubation with P13^{suc1}. The wash supernatant from the neurofilament preparation, however, containing the cdc2-like kinase, did yield cytoskeletal components that bound to P13^{suc1}. Moreover, a bacterial-expressed cdk5 associated with P13 beads, was able to complex with selected cytoskeletal components in the washed neurofilament preparation. These data indicate that direct binding of P13 beads with a cdc2-like kinase could extract active multimeric complexes composed of axonal cytoskeletal proteins and kinases.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02725-09 LNC
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Protein Phosphorylation and Regulation of Cytoskeleton in Neuronal Systems		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
P.I.	Harish C. Pant, Ph.D.	Research Chemist LNC, NINDS
Others:	Veeranna, Ph.D.	Visiting Fellow LNC, NINDS
	Alexander Wheaton	Lab Technician LNC, NINDS
	Niranjana Amin, Ph.D.	Senior Staff Fellow LNC, NINDS
	M.Takahashi, M.D., Ph.D.	Visiting Fellow LNC, NINDS
	Philip Grant, Ph.D.	Special Volunteer LNC, NINDS
COOPERATING UNITS <small>(if any)</small> S Beushausen, LN, NIH, NINDS; K Shetty, NIMHANS, Bangalore, India; M Mata, Dept. of Neurology, Univ. of Mich., Med. Center, Ann Arbor Mich., A Kulkarni, Developmental and Metabolic Branch, NINDS		
LAB/BRANCH Laboratory of Neurochemistry, BNP, DIR, NINDS		
SECTION Enzyme Chemistry		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	4.1	PROFESSIONAL: 3.6
		OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>Our process in understanding the <u>phosphorylation</u> mechanisms of <u>neurofilament protein</u>, (NF-H) is as follows: Analysis of in vivo phosphorylated sites of tail domain of rat NF-H showed that most of the Ser - residues in the Lys-Ser-Pro (KSP) repeats of rat NF-H are phosphorylated. The structural analysis of these repeat sequences suggests that multiple <u>kinases</u> are involved in their phosphorylation. One of the kinases phosphorylating KSPXXK repeats is neuronal <u>cyclin-dependent kinase-5 (cdk5)</u>. Although Cdk5 is associated with <u>G₁ cyclins (cyclin D)</u> in mitotic cells, the kinase activity is found only in mature neuronal cells. We demonstrated that, neuronal cdk5 activity is regulated by a protein of <u>67kd (P67)</u>. Peak activity correlated with the maximum levels of p67 and cdk5. p67 is neurospecific, present in both CNS and PNS neurons. It is expressed in axons of hippocampal cell cultures where it colocalized with phosphorylated NF-H (P-NF-H). In addition to its role as a <u>putative regulator</u> of cdk5, p67 is also a <u>syntaxin</u> binding protein that is thought to play a role in <u>synaptic transmission</u> and <u>secretion</u>. To further characterize the role of p67 in neural tissue, we carried out an <u>immunoblot</u> and <u>immunohistochemical</u> analysis of the developing rat <u>postnatal cerebellum</u> using antibodies to cdk5, p67, syntaxin and P-NF-H. The immunoblots showed that all antigens were developmentally regulated, increasing in expression from PN2 to the adult, with p67 and cdk5 showing a close temporal correlation. Immunohistochemically, however, cdk5 and P-NF-H showed strong colocalization whereas syntaxin and p67 antigens were tightly colocalized in regions undergoing vigorous synaptogenesis. In fiber bundles in the deep cerebellum, however, p67, cdk5 and P-NF-H were colocalized at several stages. The results suggest that p67 may have more than one function in different regions of the developing cerebellum. Recently, another regulator protein molecule <u>35kd (p35)</u>, of cdk5 has been reported but its expression is restricted to the CNS. It is not clear whether both regulator molecules are required for maximal activity, or the kinase specificity is directed to different substrates by regulators. To understand the specific roles of p67 and p35 in cdk5 regulations, we have purified large quantities of bacterially expressed cdk5, p67 and p35. A quantitative evaluation of kinase activity in the presence of p67 and or p35 and phosphorylation of different neuron specific substrates molecules with KSPXXK motifs will provide some of the answers to the above questions.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 00813-34-LNC

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Enzymological Aspects of Neural Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	R.W. Albers, Ph.D.	Section Chief	LNC, NINDS
Others:	Alexander Wheaton	Lab Technician	LNC, NINDS

COOPERATING UNITS (if any)

J P Froehlich, NIA, NIH, Baltimore; K Fendler, Max-Planck-Institut für Biophysik, FRG; K Taniguchi, Dept. of Chemistry, Hokkaido University, Hokkaido; A Dosemeci, Lab. of Neurobiology, NINDS, Bethesda

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	1.5	PROFESSIONAL:	1.0	OTHER:	0.5
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There are currently three studies within this project:

- 1) *Transient kinetics of the sodium pump*: Investigations into the cause of the biphasic characteristics of catalytic site phosphorylation and dephosphorylation, in collaborations with Froehlich, Fendler and Taniguchi.
- 2) *Regulatory modifications of the sodium pump*: Current studies are directed toward testing the hypothesis that the great sensitivity of P-type cation pumps to certain solvents is an "osmotic stress" effect on the accessibility of water to certain sites in these pump molecules.
- 3) *Regulatory modifications of calmodulin-dependent kinase II*: Studies of the functional consequences of the autophosphorylation states of calmodulin-dependent kinase II, in collaboration with A. Dosemeci.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02898-02 LNP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Physiology of CNS Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Roland Somogyi, Molecular Pharmacology Unit, Senior Staff Fellow, LNP, NINDS
 Cecilia Pazman, Fogarty Fellow
 Agnes Berki, Special Volunteer
 Osvaldo Giorgi, Visiting Scientist Volunteer
 Xiling Wen, Visiting Scientist
 Jian Wang, Special Volunteer
 Yoong Chang, Technician

COOPERATING UNITS (if any)

Laboratory of Molecular Biology, NINDS, NIH, Stroke Branch, NINDSNIH, Section of Geriatric Psychiatry, NIMH, NIH, Center for Brain Research, Faculty of Health Sciences, Ben Gurion Univ. Negev, Israel.

LAB/BRANCH

Laboratory of Neurophysiology, DIR, NINDS

SECTION

Molecular Physiology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda MD, 20892

TOTAL STAFF YEARS:

6.8

PROFESSIONAL:

3.0

OTHER:

3.8

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The studies of gene expression in development have been extended to include aspects of injury, neuroprotection and repair. To this end, we have used the model of kainate-induced seizures in rats which is accompanied by selective excitotoxic cell death in hippocampal regions. Using the quantitative RT-PCR method previously established in this laboratory, we have discovered that the developmental forms of GAD, G67I80 and G67I86 (alternatively spliced forms of GAD67 coding for two truncated proteins) are selectively and reexpressed in the hippocampus following kainate treatment. Together with evidence showing the limited expression of G67I80/86 mRNA to neurogenesis of the developing hippocampus, we have provided an indication for the recapitulation of developmental programs. *In situ* hybridization using a probe specific for G67I80/86 shows a pattern of kainate-induction which is restricted to the dentate gyrus, which is largely protected from cell death. Does this observation signify that the G67I80/86 counteracts excitotoxicity through production of the inhibitory transmitter GABA, or could this be the "tip of the iceberg" of reexpressed developmental genes that exercise a cumulative neuroprotective function? Analogously, we have employed our previously established expressed gene survey strategy, MAPP (multiplex arbitrarily primed PCR) to identify changes in gene expression in dentate gyrus following kainate treatment. We have been able to identify several differentially expressed genes, ranging from heat shock proteins, T-complex protein, the nACh receptor alpha-7 subunit to 6 novel genes. The state of maturation of fetal CNS cells directly correlates to their buoyant density, allowing the separation of cell populations using a Percoll density gradient. This is reflected in the differential expression of neurofilament and nestin mRNAs as developmental markers in different buoyant fractions. To reduce the template requirement, we have developed a direct RT-PCR technique which permits RT-PCR analysis of single 500 cell sample. By combining this technique with continuous percoll separation of naturally banding fractions of cells, we are characterizing the expression of a Multitude of genes to unequivocally define the cell types that constitute the developing CNS. We have conducted a 45-gene mRNA expression survey in the spinal cord from E12 to adult of genes which belong to the groups of (a) growth factors and their receptors (cumulatively restricted to early development); (b) neurotransmitter synthesizing enzymes and receptors (cumulatively transiently overexpressed during neurogenesis); (c) intracellular signaling genes (diverse expression patterns); and (d) developmental markers (controls). Our results show that genes in development are regulated in groups and phases, suggesting that gene expression clusters behave as units. These units are an important key to understanding function in a context that approaches the reality of cross-linked, redundant and self-stabilizing genetic networks. Robotic tools for the expression analysis of gene families in distinct cell populations are currently being established in this group and are being further explored in cooperation with the Technology Transfer Office in a CRADA.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02767-08 LNP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Image Processing and Analysis of Cellular Structures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: T.G. Smith, Jr., Senior Scientist, LNP, BNP, DIR, NINDS

Others: A.E. Schaffner, Biologist

T. N. Behar, Technician..

COOPERATING UNITS (if any)

G. D. Lange, (IACS, NINDS); W. B. Marks (LNLG, NINDS); E. A. Neale, L. M. Bowers (LDB, NICHD); Seth R. Goldstein, (BEIP, NCRR); Andreas Reichenbach, Kurt Brauer (Leipzig University, Germany); Dieter Senitz (Wurzburg University (Wurzburg, Germany); Michael Unser (BEIP, NCRR); Michael Vhrel (BEIP, NCRR); Thomas Hubin (AOSystems Design (Laurel, MD)..

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Unit on Sensory Physiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.1

PROFESSIONAL:

1.0

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued to employ the concepts of Mandelbrot's fractal geometry to the quantitative studies of central nervous system neurons, and other cell types grown in tissue culture or from whole animals. We do this by employing image processing techniques to measure the fractal dimension (D), which is a measure of the complexity of the structure under investigation. In particular, the D relates to the degree of branching (e.g., of dendrites), the ruggedness of borders and the degree of space-filling of the object of interest. We have undertaken, in separate studies, how the fractal dimension changes during the differentiation and growth of glial cells from different sources (optic nerve and brain) and neurons in tissue culture. We have found that both optic nerve and brain-derived oligodendrocytes differentiate faster and to a greater extent than do both types of astrocytes and that nerve-derived glia also differentiate faster and to a greater extent than do brain-derived glia. Interestingly, the rates of differentiation, as measured by D, can be described by a single time constant. The work on cultured spinal neurons shows that the cells can be classified into four groups on the basis of the number of their primary dendrites and that they differentiate in a similarly simple fashion, with each of the four groups having distinctive final values and time constants. We have proposed that D is a useful, quantitative measure of morphological differentiation. We examined the D's of Purkinje cerebellar cells from nine vertebrate species, ranging from birds through marsupials and mammals, including man. This indicates a phylogenetic constancy of Purkinje cell morphological complexity going back at least as far as birds in the evolutionary tree. We have begun studies of the development of the internal and surface structures of cultured rat hippocampal neurons with fluorescence and confocal microscopy in order to localize the position of GABA and glutamate bouton. We find that GABA boutons are located almost exclusively on somata and proximal dendrites, while glutamate boutons are mainly on peripheral dendrites but occasionally on proximal dendrites and less so on somata. We continue in our efforts to improve the performance of our confocal microscope with no moving parts by changes in design and components.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02608-12 LNP
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Organelle Transport of Ion Channels in Excitable Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: John R. Clay, Senior Scientist, ICB, LNP, BNP, DIR, NINDS		
COOPERATING UNITS (if any)		
A. Kuzerian (Marine Biol. Lab., Woods Hole, MA); A. Shrier (McGill Univ., Montreal, Canada); J. Trimmer (SUNY at Stony Brook, NY); K. Pfister (Univ. of VA Health Sci. Ctr., Charlottesville, VA); S. Brady (Univ. of Texas Southwest Medical Center, Dallas, TX); H. Pant (LNC)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Unit on Ion Channel Biophysics		
INSTITUTE AND LOCATION Bethesda, MD		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project concerns the transport of <u>ion channels</u> in nerve axoplasm by <u>intracellular organelles</u> . Previous work in this project has demonstrated distinct populations of organelles isolated from the axoplasm of <u>squid giant axons</u> ; a putative <u>anterograde</u> organelle population having diameters in the 40-60 nm range, and a putative <u>retrograde</u> population having diameters in the 100-150 nm range. During the past year various microscopic techniques have been applied in an attempt to visualize the organelles in various different settings. For example, <u>laser confocal microscopy</u> has recently been used to observe Brownian motion of organelles which had been labeled with the fluorescent marker, <u>Texas Red</u> . These results are being used to determine the relative yield of organelles in the various fractions from the column, and also to provide some information on organelle size from the Brownian motion. The Texas Red treated preparations are also being used in an attempt to visualize motility of exogenous organelles along <u>microtubules</u> and perhaps also <u>actin filaments</u> in an in vitro motility assay with the <u>differential interference contrast</u> microscopy technique, and they are also being pressure injected into squid axons to determine the targeting of organelles to the axonal membrane with a <u>fluorescence microscope</u> .		



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS-02631-12 LNP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function in Retinal Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: R. Nelson, Senior Scientist, LNP, NINDS

Yong-Xin, LI, Visiting Fellow

Others: M.A.Freed, Staff Fellow

A.E. Schaffner, Collaborative Investigator

M.K. Walton, Special Volunteer

COOPERATING UNITS (if any)

Physiology, University of Vienna, Austria (Renate Pflug); Physiology, University of Utah School of Medicine, Salt Lake City (Helga Kolb); Psychology, Queens College, City University of New York (Thomas Frumkes)

LAB/BRANCH

Laboratory of Neurophysiology, DIR, NINDS

SECTION

Neural Circuitry Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda Maryland 20892

TOTAL MAN-YEARS:

3.3

PROFESSIONAL:

3.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neural organization and neural responses in mammalian retinas are investigated using electrophysiological, optical, and pharmacological techniques: (1) GABA- and glutamatergic responses were recorded from dissociated rat rod bipolar cells (rb's) and other dissociated rat retinal neurons (round cells) using oxonol, a fluorescent, potentiometric dye. Fluorescence signals simultaneously recorded from dendrites, cell bodies, and in a few cases, axon terminals of rod-bipolar cells superimposed well. GABA (25 μ M) decreased fluorescence (~ 0.1 log unit) signaling hyperpolarization in both rb's and round cells, with bicuculline blockade evident in about 20% of cases. Kainic acid (50 μ M) decreased fluorescence, signaling hyperpolarization in rb's, while in round cells fluorescence often increased (~ 0.5 -log unit), signaling depolarization. Both effects were blocked by CNQX. Gramicidin, which depolarizes all cells to near 0 mV, increased fluorescence by 0.5 log units in both rb's and round cells. Fluorescence signals resembled those expected of voltage records. (2) Light-evoked noise in ON alpha-ganglion cells has been studied in a flat-mount, superfused cat retina. In this preparation, electrodes are aimed under visual control at alpha ganglion cells, and penetration is verified by immediate staining of impaled cells with pyranine dye. After TTX (20 nM) blockade of impulse activity, maintained, light-evoked, depolarizing generator potentials were observed, together with increased voltage variance (noise). Stepped applications of CNQX (1-10 = E6M) reduced both generator potential and associated noise variance in a manner indicating that quantal event size was also reduced. This suggests that some alpha ganglion cell dark noise, and nearly all light evoked noise, arises from glutamate quantal packets impinging directly on ganglion-cell dendrites. (3) The neural circuitry of ON-OFF amacrine cells in cat retina was studied electrophysiologically and ultrastructurally with HRP-filled microelectrodes. Stained cells were of multiple types; however, all dendritic arborizations were monostratified and consisted of two zones: a central zone of dendritic branching, and a more distal zone of multiple axon-like processes. Gaussian receptive fields were larger than central dendritic zone diameters. Reversal potentials for both ON and OFF components were positive to resting potentials. Input from amacrine and one or more cone-bipolar cell types and output onto ganglion cells was noted.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02330-18 LNP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Biological Studies of Developing CNS Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Barker, Chief
Others: A. Schaffner, Biologist D. Maric, Visiting Associate
W. Ma, Senior Staff Fellow I. Maric, Visiting Associate
T.N. Behar, Microbiologist F. Lahjouji, Visiting Fellow
S.V. Smith, Biologist

COOPERATING UNITS (if any)

SAIC, Fairfax, VA (J. Hickman); Naval Research Laboratories, Washington, DC (D. Stenger)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

6.3

PROFESSIONAL:

5.3

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Flow cytometry, buoyant density-gradient cell fractionation, dissociated cell culture, cell migration, immunocytochemistry and in situ hybridization methods are applied to embryonic/early postnatal rat CNS tissues to study the development, differentiation and cellular distribution of transmitter, transmitter-related enzymes and their corresponding receptors. During the past several years, we have focused primarily on GABA, which is transiently expressed in a widespread manner during CNS development before it becomes relatively restricted to fast-transmitting synapses in the adult where it often functions in an inhibitory manner. In FY95 we investigated the following: 1) transcripts encoding three GABA-synthesizing GAD enzymes and those encoding most GABA_A receptor subunit proteins were detected by in situ techniques in progressively more regions of the developing CNS; 2) specific subunits are expressed at all levels of the embryonic neuraxis beginning during the period of intense neuroblast proliferation; 3) distinct patterns of GAD and GABA transcript coexpressions are apparent: one almost exclusively in cells of the neuroepithelial proliferative zone, one in many, if not most differentiating cells during embryogenesis and one differentiating during the postnatal period; 4) some transcripts are only transiently detected for variable periods while others persist, becoming restricted to subpopulations; 5) transcripts encoding GAD and GABA receptors are more abundant and widely distributed during embryogenesis than in the adult, implying "morphogenic" roles; 6) GAD and GABA receptor subunit family proteins were detected in cells and processes, indicating that the transcripts are functional; 7) continuous-gradient centrifugation of embryonic CNS cells reveals that subpopulations accumulate in visible bands of specific buoyant density beginning during the logarithmic growth period; 8) FACS analyses of fractionated cells show that highly proliferative cells populate the dense region while primarily post-mitotic elements in G₀ or G₁ compose the visible bands; 9) FACS studies show that differentiation marker⁺, GAD⁺, GABA⁺, GABA_A receptor subunit⁺ cells exhibit characteristic patterns of expression according to their buoyant density; 10) GABA⁺ immunoreactivity can be eliminated by ionophore treatment, indicating dynamic lability in the immunodetectable signals; 11) FACS recordings of membrane potential and Ca_v²⁺ levels reveal characteristic patterns of functional GABA, glutamate and ACh receptor-coupled responses exhibited by different subpopulations of specific buoyancy; 12) migration/motility studies of GABA on embryonic cortical cells show that gradient-directed migration occurs at femtomolar GABA, while gradient-independent motility requires micromolar GABA; 13) structure-activity studies show that A, B and C are implicated; 14) GABA receptor subtypes almost all migration/motility stops when cells are loaded with BAPTA-AM, indicating Ca_v²⁺-dependent signal transduction; 15) GABA⁺ spinal and hippocampal neurons differentiate in culture independently of the initial presence of cortical astrocytes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02019-23 LNP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Properties Developing on CNS Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Barker, Chief, LNP, NINDS

Others: A. Schaffner, Biologist

J-M. Mienville, Visiting Fellow

Q.Y. Liu, Visiting Fellow

R. Serafini, Visiting Fellow

Y.X Li, Visiting Fellow

H. Xian, IRTA Fellow

COOPERATING UNITS (if any)

ICS, NINDS (G.D. Lange)

LAB/BRANCH

Laboratory of Neurophysiology, BNP, DIR, NINDS

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.4

PROFESSIONAL:

4.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Electrophysiological and digital video microscopic techniques are used primarily to elucidate the development, differentiation and cellular distribution of physiological properties expressed by embryonic and postnatal mammalian CNS neurons. Electrical studies involve direct, continuous high-fidelity amplification of ion fluxes generated either in single cells or patches or in pairs of cells in culture. Optical recordings include indirect measurements of membrane potential or cytoplasmic Ca^{2+} (Ca_c^{2+}) in small populations (50-100). Culture conditions (Serum, N3; poly-D-Lysine (PDL), astrocytes) are used as independent variables. Although cultured embryonic hippocampal and spinal cord neurons differentiate fast-transmitting networks, coculture with astrocytes has immediate and delayed effects. Principal findings include: 1) astrocytes immediately enhance GABA_A receptor/ Cl^- channel activation in a contact-dependent manner in the absence of any changes in unitary channel properties relative to values recorded in neurons on PDL; 2) BAPTA-AM loading of astrocytes eliminates, and coculture in elevated K^+ enhances their modulatory effects; 3) coculture immediately enhances voltage-dependent Na^+ channel activity relative to that recorded in neurons on PDL without change in activation/inactivation kinetics in a contact-dependent manner; 4) coculture significantly decreases input resistance and increases membrane capacitance after 1 day relative to values recorded in neurons on PDL in a contact-dependent manner; 5) coculture accelerates the time-dependent changes in the Cl^- gradient across the plasma membrane and these effects are mimicked by astrocyte-conditioned medium; 6) unitary Cl^- conductance increase and open-time kinetics shorten over time at the same rate with/without astrocytes; 7) spontaneous GABAergic synaptic-like transients appear in progressively more neurons at a faster rate in cocultures and these effects are partially mimicked by astrocyte-conditioned medium; 8) spontaneous glutamatergic synaptic-like transients are also accelerated in their appearance; 9) unitary open-times of GABA-activated Cl^- channels estimated in cells dissociated from spinal and supraspinal regions of the embryonic CNS varies and the absolute values correlate with *in situ* density of specific GABA_A receptor-subunit expressions two-fold; 10) GABA depolarizes gramicidin-perforated-patch-recorded embryonic neurons without triggering action potential activity, yet GABA triggers action potentials in ON-cell patch recordings of intact cells; 11) GABA triggers Ca_c^{2+} elevations in subpopulations of fractionated cortical cells in a similar proportion of cells to that recorded by flow cytometry of suspended cells; 12) GABA, but not muscimol triggers GABAergic Cl^- conductance transients at concentrations that do not themselves activate Cl^- channels; 13) GABA-induced GABAergic transients are Ca_0^{2+} dependent. pharmacologically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01659-27 LNP

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synaptic Contacts of Retinal Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Lasansky, Senior Scientist LNP, BNP, DIR, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Unit on Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During this period, the work has been concerned with obtaining whole-cell recordings from depolarizing bipolar cells in a consistent fashion. This usually requires dipping briefly the electrode tips in a protamine solution, a treatment that fails when phosphonucleotides are added to the electrode solution because they react with protamine and cancel its effect. In spite of numerous attempts to find alternative methods, the protamine treatment of the electrode remains the only effective one. Therefore, its use will be continued but provisions have been made to add the phosphonucleotides by electrode perfusion after the whole-cell mode of recording has been established.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02879-03 BFSB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistics and Neuroimaging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Gregory Campbell, Ph.D.	Chief, Analyt. Biomet. Sect.	BFSB, DIR
Others:	Nicholas Lange, Ph.D.	Special Expert	BFSB, DIR
	Alan Polis	Computer Systems Analyst	BFSB, DIR

COOPERATING UNITS (if any)

MNB, DIR, NINDS (Drs. M. Hallett, J. Grafman, M. Dalakas); NB, DIR, NINDS (Dr. J. Alger); CNB, DIR, NINDS (Dr. J. Higgins); LCE, NHLBI (P. Jezard); Univ. of Maryland, Medical School (Dr. H. Levin)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Analytical Biometrics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.65

PROFESSIONAL:

1.75

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project undertakes the development and application of statistical methodology to neuroimaging. In particular, while brain imaging is a fundamental tool in neuroscience, the statistical treatment of the quantification of such images has lagged behind imaging technology. Numerous statistical problems are just beginning to be addressed in the analysis of neuroimages. These include: design of experiments to limit the search volume in image analysis by either *a priori* knowledge or a previous scan; the analysis of voxels (volume elements) of images to investigate brain volumes of change in positron emission tomography (PET), magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) scans of the same individuals under different tasks or drugs; or of two groups of individuals; multiple comparison issues to exploit the spatial correlation and to control the experiment-wise Type 1 error of any inferences concerning a brain volume of apparent activity; techniques to analyze time-course data in functional MRI experiments; and the planning of experiments to ensure adequate power. Further, the resolution of these problems is crucial as the imaging technology continues to improve dramatically. Research has been conducted concerning receiver operating characteristic (ROC) methodology that has direct application to the evaluation of different imaging modalities. Papers have been submitted, are in review or were published in FY94 on the following topics: variability and covariability in magnetic resonance functional neuroimaging, induced ischemia in the motor areas of the brains of normal volunteers as assessed by PET scans (MNB); new computationally-intensive methodologies to evaluate ROC plots that are useful in comparison of imaging or artificial neural network modalities; a functional MRI study on cortical activation during mental calculation (MNB); statistical methodology for analysis of functional MRI data; functional MRI of motor-ideation in finger movement using time series analysis (MNB); studies of the statistical variability of metabolites in repeated MRS scans of normal volunteers and comparison of the scans of normals versus patients with cerebellar degeneration (NB); PET studies of the frequencies and the complexities of finger movements in normal volunteers using statistical methodology on the voxels (MNB); and the analysis of regional MRI volumes as function of age and gender in the brain development of children (CHP/NIMH).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02810-06 BFSB									
PERIOD COVERED October 1, 1994 through September 30, 1995											
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Statistical Coordinating Center for Collaborative Clinical Studies											
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Jonas H. Ellenberg, Ph.D.</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">BFSB, DIR</td> </tr> <tr> <td>Others: Karin B. Nelson, M.D.</td> <td>Medical Officer</td> <td>NEB, DIR</td> </tr> <tr> <td>Jack Panossian</td> <td>Programmer</td> <td>BFSB, DIR</td> </tr> </table>			PI: Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR	Others: Karin B. Nelson, M.D.	Medical Officer	NEB, DIR	Jack Panossian	Programmer	BFSB, DIR
PI: Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR									
Others: Karin B. Nelson, M.D.	Medical Officer	NEB, DIR									
Jack Panossian	Programmer	BFSB, DIR									
COOPERATING UNITS <small>(if any)</small> J. William Langston, M.D. and Caroline Tanner, M.D., California Parkinson's Foundation											
LAB/BRANCH Biometry and Field Studies Branch											
SECTION Collaborative Studies Section											
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 0.4	OTHER: 1.1									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input checked="" type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input checked="" type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews		
<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither									
<input checked="" type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK <small>(Use standard unrounded type. Do not exceed the space provided.)</small> <p>This project encompasses <u>collaborative studies</u> undertaken by the Office of the Chief. A major initiative involves the study of the <u>etiology of Parkinson's disease</u> (PD) using the <u>twin pair registry</u> of the National Academy of Sciences/National Research Council. The prevalent cases of PD in the more than 6,000 twin pairs in which both members are alive, will be identified. This study will evaluate: environmental, medical and family histories of both affected and unaffected members of the twin pairs; and measurement of progression of disease over time. This project will investigate genetic and environmental contributions and their interactions to the etiology of PD. A Cooperative Agreement was funded for the clinical aspects of this study. BFSB provided statistical coordination and data management.</p> <p>This project has been directed by Dr. Jonas H. Ellenberg. With Dr. Ellenberg's departure from NINDS, the Branch has continued to provide limited data management support for the project. International analytic surveys of neurological diseases reported under this project in FY 1994 are now reported in Project ZO1 NS 02652-11 BFSB, Statistical Collaboration and Consultation.</p>											



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02652-11 BFSB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Statistical Collaboration and Consultation		
PI: James M. Dambrosia, Ph.D. Others: Jonas H. Ellenberg, Ph.D. Paul S. Albert, Ph.D. Dallas Anderson, Ph.D. Gregory Campbell, Ph.D. Lisa McShane, Ph.D. Nicholas Lange, Ph.D.	Chief, Mathematical Statistics Section Chief Mathematical Statistician Mathematical Statistician Chief, Analytical Biometrics Section Mathematical Statistician Special Expert	BFSB, NINDS BFSB, NINDS BFSB, NINDS BFSB, NINDS BFSB, NINDS BFSB, NINDS
COOPERATING UNITS (if any) Bombay Hosp India (Dr. N. Bharucha); Peking Union Medi Coll, PRC (Dr. Z. Zhang); NIMH; Dr. N. Rosenthal; Inst Stroke Re and Prev, Austria (Dr. M. Brainin); Mt Siani Med Cent N.Y. (Dr. S. Tuhirim), Univ. Md. Sch Med (Drs. S. Kittner & T. Price), Reg Hosp, Junin, Argentina (Dr. Mario Melcon)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Office of the Chief, Mathematical Statistics Section, Analytical Biometrics Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.6	PROFESSIONAL: 2.0	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input checked="checked" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project encompasses a wide scope of statistical collaboration and consultation with laboratories and branches within the Division of Intramural Research (DIR), and with other neuroscience units outside NIH. Particular consideration is given to <u>statistical planning and design of experiments</u> , <u>statistical analysis of data</u> , and <u>statistical inference</u> . Examples of current studies include: clinical trial of bioequivalence of α -mannose-terminated glucocerebrosidase from natural and recombinant sources, clinical course and long-term outcome of patients treated with Ceredase TM Gaucher' evaluation of skeletal responses of Gaucher patients treated with Ceredase TM (DMNB); identification of factors associated with post-operative status of neurosurgical patients after trans-sphenoidal pituitary surgery (SNB); PET measurement of the effect of time from last seizure and seizure type on metabolic change, prevalence study of neurologic diseases in the Navajo tribe (ERB); modeling lesion recurrence in relapsing-remitting MS, clinical trial of DSG on lesion development in relapsing-remitting MS, monitoring MRI T2 weighted imaging in relapsing-remitting MS; examining the effect of accumulation of disease white matter in patients with relapsing-remitting MS (NIB); study of abnormal facilitation response to transcranial magnetic stimulation in PD patients, identification of deficits associated with "over use" syndrome in pianists, two clinical trials of IV/IG in neuromuscular disorders, follow-up study of twins discordant for paralytic polio and subsequent post- polio syndrome; clinical trial of amantadine for the treatment of post-polio fatigue clinical trail of buspirone for the treatment of cerebellar ataxia; classification of MSA, PAF, and healthy individuals based on electrophysiological tests of autonomic function (MNB); validation study of consultations provided by U.S. drug information centers (CC); incidence study of nervous system tumors in Israel; incidence study of motor neuron disease on Guam, population-based case-control study of electronic fetal monitoring abnormalities as risk factors for CP in children weighing more than 2500g; comparison of risk factors for CP in low birthweight versus term birthweight children; planning and design of a clinical trial of magnesium sulfate for the reduction of CP in premature births (NEB); development of Markov models for rapidly cycling bipolar disorder(NIMH); studies of silent stroke risk factors for a subsequent stroke; recursive partitioning and logistic modeling for determination of risk of a cardiac source of embolism for ischemic stroke stroke.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02490-15 BFSB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Research in Statistics		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	James M. Dambrosia, Ph.D.	Acting Chief BFSB, DIR
Others:	Jonas H. Ellenberg, Ph.D.	Chief BFSB, DIR
	Dallas W. Anderson, Ph.D.	Mathematical Statistician BFSB, DIR
	Gregory Campbell, Ph.D.	Chief, Analytical Biometrics Section BFSB, DIR
	Lisa M. McShane, Ph.D.	Mathematical Statistician BFSB, DIR
	Paul S. Albert, Ph.D.	Mathematical Statistician BFSB, DIR
	Nicholas Lange, Ph.D.	Special Expert BFSB, DIR
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	1.2	PROFESSIONAL: 1.2 OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project addresses statistical problems generated from collaboration with scientists in other program areas and general statistical problems of current interest. his project is a continuing activity of the Section on Mathematical Statistics and other members of the Branch. In FY 1995, Branch statisticians have contributed to the following areas of statistical research: evaluation of proxy respondents and auxiliary information as adjustments for nonresponse or attrition in disease surveys ; modeling seasonal change in time series regression relationships; development of both discrete and continuous time Markov models for longitudinal categorical data which accounts for different processes across subjects; derivation of statistical methods for the detection of and tests of differences among spatial disease clusters; estimation of selection bias and its effect on inferences from observational data bases; development of adjustment methods to account for differential mortality for the evaluation of factors associated with increased disease risk. Other work includes: methods to improve coverage in surveys; estimation of time-to-event data with interval censoring; site selection for epidemiologic surveys; analysis of response surface data with spatial and temporal components; modeling of response surfaces with spatially correlated errors; application of splines to estimate model parameters of multiple correlated response surfaces; modeling effect changes of covariates in the presence of spatial correlation; analysis of bioequivalence trials with multiple, nonlinear responses to treatment; combining information from negatively correlated nonlinear regressions; development of a generalized estimating equation approach for the analysis of spatially dependent binary data; application of bootstrap methods to longitudinal natural history data; use of variance component methods to assess the precision of biochemical measurements; using a Markov chain model to study three state disease processes; evaluation of case ascertainment strategies for area surveys of neurological diseases; and sampling strategies for spatial point processes with multiple types of clustering. </p>		



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02914-01CNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Imaging of Neurotransmitter Function with Positron Emission Tomography

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	David S. Goldstein, M.D., Ph.D.	Chief, CNS	CNB, NINDS
Others:	Courtney Holmes	Medical Technologist	CNB, NINDS
	Irwin J. Kopin, M.D.	Chief	CNB, NINDS
	John Stuhlmuller, M.D.	Senior Staff Fellow	CNB, NINDS

COOPERATING UNITS (if any)

Clinical Center PET Department, NIH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Clinical Neurochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:	1.4	PROFESSIONAL:	1.3	OTHER:	0.1
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Thoracic positron emission tomographic (PET) scanning after systemic administration of 6-[¹⁸F]fluoro-dopamine ([¹⁸F]-6F-DA), which we developed to image regional sympathetic innervation and function, is being applied to examine the pathophysiological involvement of myocardial sympathetic nerves in patients with neurologic, cardiac, or psychiatric disorders and to elucidate clinical mechanisms of action of drugs. From time-activity relationships after administration of classical neuropharmacological probes, we obtained evidence that [¹⁸F]-6F-DA PET scanning can assess not only cardiac sympathetic innervation but also specific aspects of cardiac sympathoneural function. Patients with peripheral autonomic failure (PAF) had no myocardial [¹⁸F]-6F-DA-derived radioactivity, indicating markedly decreased density or absence of myocardial sympathetic nerve terminals. One PAF patient had absent cardiac norepinephrine (NE) spillover and no cardiac PAF [¹⁸F]-6F-DA-derived radioactivity, despite normal total body and forearm NE spillover. This suggests that the neurodegenerative process in PAF can include regionally selective loss of sympathetic terminals. Patients with the Shy-Drager syndrome (SDS) had increased cardiac [¹⁸F]-6F-DA-derived radioactivity, indicating intact cardiac sympathetic nerve terminals. The rate of decline of the ¹⁸F content was slower than normal, suggesting decreased or absent nerve traffic, yet the same patients had normal or increased cardiac norepinephrine spillover. Normal cardiac NE spillover in SDS patients suggested constitutive neurosecretion as the basis for maintenance of approximately normal supine plasma NE levels in these patients. This is the first indication of constitutive neurosecretion in a neurological disease. One patient with advanced SDS had no cardiac [¹⁸F]-6F-DA-derived radioactivity, suggesting that the disease can progress to involve peripheral as well as central neurons. In patients with hypertrophic cardiomyopathy (HCM), the overgrown myocardium possesses sympathetic terminals. Several patients had regionally heterogeneous sympathetic innervation, as judged by [¹⁸F]-6F-DA-derived radioactivity in the hypertrophic myocardium and mismatches between perfusion, as indicated by ¹³N-ammonia PET scanning, and sympathoneural images. This might be related to the risk of cardiac arrhythmias in these patients and suggests that interactions between neural and myocardial growth factors contribute to development of the disease. A patient with pain due to reflex sympathetic dystrophy had evidence for decreased sympathetic innervation of the affected limb; the results agreed with those in a rat model of hyperalgesia, where arteriovenous increments in plasma NE levels were smaller on the affected than intact side. The findings therefore indicate that reflex sympathetic dystrophy actually does involve the sympathetic nervous system.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02910-02 CNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Distribution and Possible Function of Cannabinoid Receptors*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: N.E. Buckley, Ph.D. IRTA Fellow CNB, NINDS
Others: Eva Mezey, M.D., Ph. D. Visiting Scientist CNB, NINDS

COOPERATING UNITS (if any)

Andreas Zimmer, Ph.D., Visiting Associate, LCB, NIMH; Tom Bonner, Ph.D., Research Biologist, LCB, NIMH.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Aminergic Mechanisms Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.9

PROFESSIONAL:

0.9

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Marijuana (*Cannabis sativa*) has been known for many centuries as a psychoactive agent and also for its medicinal properties. In humans, it has been used as an analgesic, anti-inflammatory, immunosuppressant and anticonvulsant. Recently, two cannabinoid receptors have been cloned, the central and the peripheral cannabinoid receptors. The central cannabinoid receptor (CB1) was first found in the brain, but also occurs in the testis. The peripheral cannabinoid receptor (CB2) seems to be predominantly expressed in the spleen. This may explain the immunosuppressive properties of marijuana and its active component, delta-9-tetrahydrocannabinol (THC). Previous work has suggested that CB2 is present in the marginal zone of the spleen, but there has been discrepancy as to its cellular localization. We used specific immunocytochemistry in combination with *in situ* hybridization, as well as RT-PCR on isolated B lymphocytes, and have determined that the B cells express CB2 mRNA (manuscript submitted for publication). We have also found CB2 mRNA in testis and are currently studying whether there is a spermatogenesis-stage-specific expression of CB2 mRNA. In rat, spermatogenesis consists of 14 stages which can be synchronized with a vitamin A deficient diet. We are beginning to study the levels of CB2 mRNA in the synchronized testis. Thus, we may better understand the role of CB2 in spermatogenesis. The expression of CB1 or CB2 during prenatal development is unknown. We have done *in situ* hybridization in different aged embryos using probes for CB1 and CB2 mRNA, and found differential expression of each of the receptors as early as day 11 after conception. To study the significance of the presence CB2 in an organism, we will produce transgenic mice lacking the peripheral cannabinoid receptor. We have successfully cloned the mouse CB2 receptor, sequenced the coding sequence, and prepared the plasmid construct for homologous recombination. We have successfully obtained eight clones of embryonic stem cell in which the gene for CB2 was disrupted by homologous recombination. We are now in the process of introducing each of these eight clones into embryos to produce the transgenic mice.

*Formerly entitled, "Localization of Cannabinoid Receptors".

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02883-03 CNB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Peripheral and Central Nervous System Peptide Neurotransmitters*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Eva Mezey, M.D., Ph. D.	Visiting Scientist CNB, NINDS
Others:	Gyongyi Harta, B.A.,	Visiting Associate CNB, NINDS
	Nancy Buckley, Ph.D.	IRTA Fellow CNB, NINDS
	Krisztina Krempels, M.D.	Visiting Fellow CNB, NINDS
COOPERATING UNITS (if any) Graeme Eisenhofer, CNB, NINDS; Miklos Palkovits, M.D., Ph.D., Visiting Scientist, LCB, NIMH; Beth Hoffman, Ph.D., Senior Staff Fellow, LCB, NIMH; Bela Hunyady, LCB, NIMH		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Neuroanatomy/Aminergic Mechanisms		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS:	3.9	PROFESSIONAL: 3.1 OTHER: 0.8
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%; text-align: center;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%; text-align: center;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>We have completed the work on the localization of the <u>cannabinoid type 2 receptor</u> in the immune system and found that the receptors are present in B cells of the marginal zone in the spleen. This finding might explain the well known effects of marijuana on immune function and suggests the possible use of specific cannabinoid 2 antagonists to boost immunoglobulin production or the use of agonists to suppress immune function (in the cases of tissue transplantation for instance). A transgenic mouse that would lack the receptor for the cannabinoid 2 receptor has been successfully completed. Several constructs have been injected into stem cells and the mice (if they are viable) will be available in the near future.</p> <p>We have successfully mapped all <u>somatostatin receptors</u> in the pituitary gland and colocalized their mRNA with the different pituitary hormones. We also finished a study on the presence of receptors of the VIP family (VIP1, VIP2 and PACAP) in the rat testis and concluded that the well known effects of PACAP in the testis are due to its binding to the type 2 VIP receptor, and not the PACAP receptor - as previously thought. We are in the process of mapping and studying the distribution of somatostatin receptors in the peripheral tissues.</p> <p>Work has continued on the mechanisms involved in ulcer formation in the GI tract. We discovered that the parietal cells of the stomach are positive for tyrosine hydroxylase (TH) and dopamine. In collaboration with Graeme Eisenhofer we found very high concentrations of dopamine in the gastric juice. With the help of Beth Hoffman and Bela Hunyady (NIMH, LCB) they have succeeded in isolating gastric mucosal cells, then measuring dopamine in these cells, and also quantitating TH activity in the superficial mucosal layer (GE). <i>In situ</i> hybridization histochemistry shows that the dopamine 1b (= D5) receptor is present in most epithelial and many lamina propria cells in the stomach and can be the target of this locally made dopamine. In this new non-neuronal dopamine system, dopamine might act as a hormone, since it is secreted into the stomach, and is stable in an acidic environment.</p> <p>*Formerly entitled, "Distribution and Role of Neurotransmitters and Their Receptors".</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02870-04 CNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Amines: Regulation and Function*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	I. J. Kopin, M.D.	Chief, CNB, Director, DIR	CNB, NINDS
Others:	S. Al-Damluji, M.D.	Visiting Scientist	CNB, NINDS
	K. Pacak, M.D.	Visiting Fellow	CNB, NINDS
	Gal Yadid, Ph.D.	Visiting Fellow	CNB, NINDS
	J. Harvey-White, B.S.	Technician	CNB, NINDS
	D.S. Goldstein, M.D., Ph.D.	Medical Officer	CNB, NINDS
	Y.F. Duan, Ph.D.	IRTA Fellow	CNB, NINDS

COOPERATING UNITS (if any)

Carl Hart, B.S., IRTA Fellow; Richard McCarty, Ph.D., Special Volunteer; Eva Mezey, Ph.D., Visiting Scientist, CNB, NINDS; Miklos Palkovits, M.D., Ph.D., LCB, NIMH.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Aminergic Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.9

PROFESSIONAL:

2.9

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objectives of this project are to determine the roles of catecholamines in brain functions that regulate motor activity, neuroendocrine secretion, and autonomic function. *In vivo* microdialysis is used to monitor levels of monoamines and their metabolites in extracellular fluid in specific brain regions, with simultaneous measurements of arterial plasma levels of norepinephrine (NE), epinephrine (EPI), and ACTH. Microdialysis probes with an attached infusion microcannulae are used to introduce drugs or isotopically-labelled compounds and sample extracellular fluid in the same region. Brain lesions are made surgically or by use of toxins specific to particular cell types. HPLC and liquid scintillation spectrophotometry are used in assays of materials in the microdialysates and *in situ* hybridization used to examine brain regional levels of mRNA encoding CRH. In rats with unilateral striatonigral dopamine (DA) neurons destroyed with 6-OHDA, DA formation from locally perfused DOPA was detected but was less on the lesioned than on the intact side. Clorgyline (an inhibitor of MAO-A), augmented DA production from DOPA both on the lesioned and the intact side, whereas deprenyl (an inhibitor of MAO-B) had no effect. Neuroendocrine responses to a variety of stressors (handling, immobilization, subcutaneous formalin injection, insulin, hemorrhage, or cold) indicated stressor-specific patterns. For example, insulin-induced hypoglycemia evoked marked, correlated increases in EPI and ACTH levels, whereas cold exposure increased plasma NE levels disproportionately compared with ACTH responses, and hypotensive hemorrhage increased ACTH levels disproportionately compared with catecholamine responses. Administration of cortisol inhibited basal and immobilization stress-induced NE release and catecholamine synthesis in the paraventricular nucleus (PVN). After adrenalectomy, release was enhanced, and the effects of adrenalectomy were reversed by treatment with replacement doses of cortisol. Thus, central noradrenergic pathways ascending from medullary centers participate in feedback inhibition of HPA axis activity exerted by circulating glucocorticoids. After brainstem hemisection, which interrupts noradrenergic pathways from the A1 and A2 areas to the PVN, immobilization stress-induced NE release in the ipsilateral PVN was reduced much more than in the contralateral PVN, whereas NE release in the central nucleus of the amygdala was unaffected on either side. Both basal levels and stress-induced enhancement of PVN mRNA encoding CRH were diminished ipsilateral to the hemisection. Plasma ACTH responses to immobilization, however, were uninfluenced by brainstem hemisection. This indicates that there is sufficient CRH release from only one PVN to elicit an unimpaired ACTH response to immobilization.

*Formerly entitled, "Regional Brain Catecholamine Release and Disposition and Responses to Stress".



NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02839-05CNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sympathoadrenal and Catecholaminergic Function in Health*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	David S. Goldstein, MD, PhD	Chief, CNS	CNB, NINDS
Others:	Graeme Eisenhofer, Ph.D.	Visiting Associate	CNB, NINDS
	Courtney Holmes	Medical Technologist	CNB, NINDS
	Irwin J. Kopin, M.D.	Chief	CNB, NINDS
	Jacques Lenders, M.D., Ph.D.	Visiting Scientist	CNB, NINDS
	John Stuhlmuller, M.D.	Senior Staff Fellow	CNB, NINDS

COOPERATING UNITS (if any)

N. Andrews; F. Axelrod; A. Breier; R. Cannon; P. Chang; V. Convertino; M. Dalakas; A. Golczynska; R. Dionne; E. Grossman; A. McRae; S. Milstien; E. Peles; A. Quyyumi; S. Raja; O. Smith; J. Vernikos

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Clinical Neurochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:

2.4

PROFESSIONAL:

1.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Distinctive neurochemical patterns associated with specific abnormalities in catecholamine biosynthesis, storage, release, disposition, and metabolism were described in several genetic or acquired diseases. Children with Menkes' disease, a disorder of copper metabolism, had evidence for decreased activity of dopamine- β -hydroxylase (DBH), with DOPA: dihydroxyphenylglycol (DHPG) ratios invariably increased, enabling *in utero* diagnosis and early treatment. Dihydropteridine reductase (DHPR) deficiency causes an atypical form of phenylketonuria. Our finding of low but detectable levels of DOPA and other catechols in a patient with absent DHPR implies that in humans, DHPR is not absolutely required for catecholamine synthesis. Patients with familial dysautonomia (FD) had a characteristic, distinct neurochemical phenotype, with high ratios of plasma DOPA:DHPG and normal plasma NE, DA, and dihydroxyphenylacetic acid (DOPAC) levels. The phenotype predicts a mutation that produces arrested differentiation of peripheral catecholaminergic systems. Patients with inherited deficiency of MAO-A had very low levels of DHPG, whereas patients with deficiency of MAO-B did not, providing a means to distinguish neurochemically deficiencies of the two isoforms of the enzyme. Plasma levels of free (unconjugated) metanephrines diagnosed pheochromocytoma better than did any other neurochemical test. Several studies assessed catecholaminergic neurochemical correlates of physiological and pathophysiological states or drug treatments. A study combining direct sympathetic nerve recording with neurochemical methods provided the first evidence for glucocorticoid-induced sympathoinhibition in humans, indicating a potentially important interaction between two of the body's main stress effector systems. Prolonged head-down bed rest was used as a model of chronic exposure to zero-gravity during space flight. Neurochemical findings indicated that chronic sympathoinhibition accompanies the orthostatic intolerance that always occurs during re-exposure to the earth's gravity. A characteristic neurocirculatory pattern was found to precede neurocardiogenic syncope, with blunted increases in forearm NE spillover during nonhypotensive LBNP and augmented plasma epinephrine (EPI) responses. In a patient with the Shy-Drager syndrome and multiple myeloma, *in vitro* testing supported an autoimmune causal mechanism for the disease. Results of a collaborative study of clozapine indicated that this novel neuroleptic affects several aspects of peripheral noradrenergic function. We obtained evidence for functional stimulatory β -adrenoceptors on sympathetic terminals in the human forearm, without evidence for functional stimulatory receptors for angiotensin II. In humans, the main identified modulator of transmitter release from sympathetic nerves appeared to be inhibitory, mediated by α 2-adrenoceptors.

*Formerly titled "Clinical Studies of Catecholamine Metabolism and Disposition"



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02752-08 CNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Synthesis and Expression of Neurotrophic Factors*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Joan Schwartz, Ph.D.	Chief, Molecular Genetics Section	CNB, NINDS
Others:	Takayuki Taniwaki, M.D.	Visiting Fellow	CNB, NINDS
	Yukihiro Sugita, M.D., Ph.D.	Visiting Associate	CNB, NINDS
	Vivian Wu, Ph.D.	Visiting Fellow	CNB, NINDS
	Takehisa Araki, M.D.	Visiting Fellow	CNB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

4.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	<input type="checkbox"/>	(b) Human tissues	<input checked="" type="checkbox"/>	(c) Neither
<input type="checkbox"/>	(a1) Minors				
<input type="checkbox"/>	(a2) Interviews				

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evidence suggests that parallel biochemical and regulatory processes occur during normal development and following various forms of central nervous system (CNS) injury. Among these, areas of particular interest are: (1) identification of CNS neurotrophic factors; and (2) the analysis of the regulation of neurotrophic factor and neuropeptide gene expression during development and in response to injury. Studies are underway to identify trophic factors produced in specific model systems, since recent evidence demonstrates the existence of numerous different neurotrophic factors. 6-OHDA-lesioned rats represent a Parkinsonian-like model in which changes in nerve growth factor, brain-derived neurotrophic factor and NT-3 are being examined at the level of mRNA, protein, and biologic activity. Since astrocytes can synthesize a number of neurotrophic factors, primary cultures of astrocytes are used to determine factors which regulate production of these potential trophic factors. Reactive astrocytes are prepared from 6-OHDA-lesioned brain: monoclonal antibodies raised against epitopes expressed only by reactive astrocytes *in vivo* can distinguish between normal adult and reactive astrocytes in culture. Astrocytes from newborn animals more closely resemble reactive astrocytes in terms of expression of these epitopes. Production of trophic factors by these reactive astrocytes is compared to that of control astrocytes to determine how injury may alter the regulatory pathways. Cytokines, produced at high levels in injured brain, induce expression of neurotrophic factors as well as of nitric oxide synthase, which may be responsible for some of the neuronal damage. Depletion of the cytokine interleukin-3 (IL-3) by an antisense construct results in transgenic mice expressing a neurologic syndrome. The lesion results from migration/activation of microglia in the cerebellar peduncle. Among novel enkephalin and somatostatin, both produced by astrocytes, have been demonstrated in culture as well as in transgenic mice. Enkephalin acts as a negative modulator of CNS development while somatostatin is a positive trophic factor. A retinal pigment epithelium-derived factor (PEDF) not only functions as a survival factor for cerebellar granule cell neurons but also can protect them against both glutamate toxicity and apoptotic cell death. In addition, PEDF activates microglia, which produce an as yet unidentified factor which inhibits astrocyte proliferation and may thus be useful in situations of gliosis due to astrocyte division.

*Formerly entitled, "Origins and Roles of Neurotrophic Factors".

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02717-10 CNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regional Peripheral Catecholamine Release and Disposition in Health and Disease*

P.I.:	Graeme Eisenhofer, Ph.D.	Visiting Associate	CNB, NINDS
Others:	David S. Goldstein, M.D., Ph.D.	Chief	CNB, NINDS
	Douglas Hooper, B.S.	Chemist	CNB, NINDS
	Judy Harvey-White	Bio Lab Technician	CNB, NINDS
	Eva Mezey, Ph.D.	Head, Neuroendocrinology Unit	CNB, NINDS
	Karel Pacak, M.D., Ph.D.	Visiting Fellow	CNB, NINDS

COOPERATING UNITS (if any)

H. Keiser, M.D., NHLBI; M. Esler, M.D., D.L. Murphy, M.D., Ph.D., NIMH; G. Lambert, B.S., Australia; P. Friberg, M.D., B. Rundquist, M.D., A. Aneman, M.D. Goteborg, Sweden; J. Lenders, M.D., Netherlands; E. Schomig, Ph.D., HdIbg, Germany

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Unit on Preclinical Neurochemistry

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.2

PROFESSIONAL:

1.1

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project centers on examination of how the sympathetic nervous system and other catecholaminergic systems function to integrate and regulate physiological processes at subcellular, cellular, and multi-organ levels in health and disease. Studies of patients with chromosomal deletions affecting monoamine oxidase, an enzyme responsible for metabolism of catecholamines, revealed three separate genetic defects affecting the enzyme. The three disorders are associated with distinct neurochemical, behavioral, & neurological phenotypes. The findings provide insight into how the enzyme functions in catecholamine metabolism, as well as how abnormalities of the enzyme may lead to developmental abnormalities in the expression of behavior. Measurement of plasma free (unconjugated) metanephrines, O-methylated metabolites of catecholamines, in patients with pheochromocytoma indicated that these metabolites provide a considerably more sensitive marker of the tumor than conventional measurements of plasma catecholamines or urinary metanephrines. Findings that large amounts of metanephrines are produced within the adrenals provide an explanation for the extraordinary sensitivity of plasma levels of these metabolites for diagnosis of pheochromocytoma; even when not actively secreting catecholamines the tumors are actively metabolizing catecholamines to metanephrines. Studies of cardiac sympathetic function in congestive heart failure indicated that increased sympathetic drive to the failing heart is secondary to both an increase in neuronal release of transmitter and a decrease in the efficiency of transmitter reuptake. Examination of patients at various stages of heart failure revealed that the changes in cardiac sympathetic function precede changes elsewhere in the body. Studies of how the handling of catecholamines is integrated among different organs and tissues of the body indicate that the liver removes most of the norepinephrine and its metabolites that are released by upstream mesenteric organs. This revealed that a previously unrecognized major proportion of sympathetic outflow is directed to mesenteric organs. Substantial dopamine production by mesenteric organs also suggests the presence of a novel dopaminergic autocrine/paracrine system within the gastrointestinal tract. The system may be involved in bicarbonate secretion and absorption of sodium, functions that may be relevant to several disorders such as salt-sensitive hypertension, idiopathic edema, and ulcer formation.

*Formerly entitled, "Catecholamine Metabolism in Health and Disease".

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02916-01

DMNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Therapy of Fabry Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. O. Brady, M.D.	Branch Chief	DMNB	NINDS
Others:	G. J. Murray, Ph.D.	Special Expert	DMNB	NINDS
	A. B. Kulkarni, Ph.D.	Special Expert	DMNB	NINDS
	J. A. Medin, Ph.D.	IRTA	DMNB	NINDS
	J. M. Quirk, M.S.	Biochemist	DMNB	NINDS
	N. W. Barton, M.D.,	Section Chief	DMNB	NINDS
	(Continued below)			

COOPERATING UNITS (if any)

Laboratory of Cell Biology, NCI

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.35

PROFESSIONAL:

1.05

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fabry disease is an X-linked inherited recessive metabolic disorder caused by insufficient activity of the lysosomal enzyme alpha-galactosidase A (ceramidetrihexosidase). In contrast with the remarkable benefit of enzyme replacement therapy developed by DMNB for patients with Gaucher disease, little progress has been made concerning specific treatment for patients with Fabry disease.

We propose to develop gene replacement therapy for patients with this metabolic disorder. Retroviral vectors containing the cDNA of human alpha-galactosidase A will be produced, and the efficiency of transduction of patients' hematopoietic stem cells will be determined. When a vector with sufficient activity and titer is available, we shall develop a Phase I gene therapy trial for patients with Fabry disease.

Additional Investigators:

S. Karlsson, M.D., Ph.D.
T. Ohshima, M.D., Ph.D.
M. M. Gottesman, M. D.

Section Chief
Visiting Fellow
Laboratory Chief

DMNB NINDS
DMNB NINDS
LCB NCI

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02893-02

DMNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modification of Brain-Specific Cyclin-Dependent Kinase Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	A. Kulkarni, Ph.D.	Unit Chief	DMNB	NINDS
Others:	T. Oshima, M.D. Ph.D.	Visiting Fellow	DMNB	NINDS
	G. Longnecker, B.S.	Biologist	DMNB	NINDS
	H. Pant, Ph.D.	Section Chief	LNC	NINDS
	R. Brady, M.D.	Branch Chief	DMNB	NINDS

COOPERATING UNITS (if any)

LNC, NINDS

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Unit on Mouse Genetics and Human Disease Models

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.7

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	<input type="checkbox"/>	(b) Human tissues	<input checked="" type="checkbox"/>	(c) Neither
<input type="checkbox"/>	(a1) Minors				
<input type="checkbox"/>	(a2) Interviews				

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Members of cdc 2 family of protein kinases are known for their pivotal role in the regulation of the eukaryotic cell cycle. The potential roles of neuronal specific cdc2-like kinases in stabilizing the neurofilament skeleton and in axonal morphogenesis through phosphorylation of neurofilament and tau are not well delineated. The main objectives of this project are (1) to disrupt the genomic locus of neuronal cdc2-like kinase (cdk5) in embryonic stem cells and then use the targeted cells to generate mouse models to study *in vivo* function of these kinases; and (2) to overexpress cdk5 in neuronal cell lines and *in vivo* in mice. We have characterized cdk5 genomic clones isolated from 129/svj library. A gene targeting construct has been engineered by deletion of exons 3 through 5 and insertion of neomycin-resistance gene at the site of deletion. At the 3' end of the construct, herpes virus thymidine kinase gene is included for positive/negative selection using G418 and gancyclovir. We have also isolated a murine cDNA clone using RT PCR technique and obtained complete nucleic acid sequence data.

We have successfully disrupted the cdk5 locus in ES cells and generated F1 heterozygous mice. In addition, we have obtained cdk5 null ES cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02894-02

DMNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Generation of Mouse Models of Neurological Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Kulkarni, Ph.D.	Unit Chief	DMNB	NINDS
Others:	K. Yoshida, M.D., Ph.D.	Visiting Fellow	DMNB	NINDS
	T. Oshima, MD, Ph.D.	Visiting Fellow	DMNB	NINDS
	G. Longnecker, BS	Biologist	DMNB	NINDS
	C. Murray, Ph.D.	Special Expert	DMNB	NINDS
	E. Cleaveland, B.S.	Molecular Biologist	DMNB	NINDS
	R. Brady, M.D.	Branch Chief	DMNB	NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Unit on Mouse Genetics and Human Disease Models

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

1.7

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gene knock-out mouse models have become gold standards for delineating molecular and functional roles of specific genes. In an attempt to generate such models for neurological disorders, we have initiated studies to disrupt apolipoprotein D (ApoD) gene in mouse embryonic stem cells. We have used rat ApoD cDNA probe to screen the 129/svj mouse genomic library. Eight genomic clones of ApoD have been isolated and characterized. An ApoD targeting construct has been engineered for positive/negative selection. ApoD cDNA has been cloned by RT-PCR from mouse kidney RNA. We expect to produce an ApoD knock-out mouse in the near future in order to determine the role of ApoD in nerve regeneration as well as its potential role in breast and prostate cancer.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02878-03
DMNB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models for Genetic Defects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	S. Karlsson, M.D., Ph.D.	Acting Chief, M&MGS	DMNB	NINDS
Others:	C.-G. Huh, Ph.D.	IRTA Fellow	DMNB	NINDS
	A. Kulkarni, Ph.D.	Senior Staff Fellow	DMNB	NINDS
	G. Longenecker, B.S.	Biologist	DMNB	NINDS

COOPERATING UNITS (if any)

P. Loh, NICHD, NIH

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

1.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	<input type="checkbox"/>	(b) Human tissues	<input checked="" type="checkbox"/>	(c) Neither
<input type="checkbox"/>	(a1) Minors				
<input type="checkbox"/>	(a2) Interviews				

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gene targeting in embryonic stem (ES) cells is being used to inactivate (knock-out) genes and use the mutated ES cells to generate mice with a mutation at the targeted locus. The cystatin C gene has been cloned and used to make gene targeting constructs. Cystatin-C knock-out mice have been made. These mice do not produce any mouse Cystatin C RNA or protein. They breed normally, but are less aggressive than normal mice and have reduced horizontal movement activity. Further pathological and neurological evaluation is in progress. Human mutant Cystatin C knock-in mice are also being developed in order to make an animal model for hereditary stroke. The knock-in is done with a mutant Cystatin C gene from a patient with hereditary cerebral angiopathy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02843-04
DMNB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of the Etiology of Mucopolidosis IV

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.Goldin, Ph.D. Visiting Fellow DMNB NINDS

Others: R.O. Brady, M.D. Chief DMNB NINDS
P.G. Pentchev, Ph.D. Section Chief DMNB NINDS
N.W. Barton, M.D., Ph.D. Section Chief DMNB NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Cellular and Molecular Pathophysiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.05

PROFESSIONAL:

1.05

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed and validated a novel test for the prenatal diagnosis of mucopolidosis IV. The phenotypic alteration that permitted the development of this test is being used as a marker for the functional cloning of the gene that is mutated in mucopolidosis IV. In addition, we have designed a selection procedure for ML4 cells based on their increased sensitivity to chloroquine over normal control fibroblasts. This procedure will allow us to rapidly isolate complementing genes in ML4 cells transfected with DNA libraries. The increase in chloroquine sensitivity also provides a clue to the nature of the abnormal cell biology in patients with ML4.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02845-04
DMNB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of Enzyme Replacement Therapy in Disorders That Affect the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	R. O. Brady, M.D.	Chief	DMNB NINDS
Others:	G.J. Murray, Ph.D.	Special Expert	DMNB NINDS
	J.M. Quirk, M.S.	Biochemist	DMNB NINDS
	G. C. Zirzow, B.S.	Biologist	DMNB NINDS
	C. Kaneski, B.S.	Biologist	DMNB NINDS
	U. Schuler, M.S.	Special Volunteer	DMNB NINDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, NINDS

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:

1.15

PROFESSIONAL:

0.25

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Enzyme replacement therapy has been shown to be extraordinarily effective for patients with Type 1 (non-neuronopathic) Gaucher's disease. We are now developing procedures to deliver useful amounts of enzymes to the brain in patients with hereditary metabolic storage disorders. We have examined the effect of human placental beta-galactosidase on the amount of ganglioside GM1 in animal analogues of human generalized (GM1) gangliosidosis using a new intracerebral protein delivery system. We are also determining the distribution of glucocerebrosidase in the brain using convection-enhanced intracerebral injection of this enzyme. We are characterizing the receptors involved in the uptake of glucocerebrosidase and other enzymes by neurons in tissue culture.



NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modification of Growth Factor Genes by Gene Targeting

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Kulkarni, Ph.D.	Senior Staff Fellow	DMN	NINDS
Others:	S. Karlsson, M.D., Ph.D.	Acting Section Chief	DMN	NINDS
	L. Yaswen, Ph.D.	IRTA	DMN	NINDS
	C.-G. Huh, Ph.D.	IRTA	DMN	NINDS
	G. Longenecker	Biologist	DMN	NINDS
	S. Yuspa, M.D.	Chief	LCCTP	NCI

COOPERATING UNITS (if any)

Stroke Branch, NINDS; LCCTP, NCI, Frederick Cancer Research & Development Center, NCI

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:

0.85

PROFESSIONAL:

0.65

OTHER:

0.20

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gene targeting by homologous recombination can be used to activate or inactivate cellular genes in eukaryotic cells. The objective of this project is to alter the functional status of genes that control growth and maturation of specific tissues and study the biological consequences of these molecularly defined alterations. We have initiated this program with an investigation to delineate specific roles of transforming growth factor beta-1 (TGF-beta 1). We have generated TGF-beta 1 knockout mice using gene targeting technique. These mice are deficient in this growth factor. After normal growth for the first two weeks, they develop a rapid wasting syndrome and die as early as three weeks of age. Histopathological examination revealed multifocal inflammatory response with massive infiltration of lymphocytes and macrophages in many organs, but primarily in heart and lungs. Further examination indicated that onset of multifocal inflammation follows elevated expression of major histocompatibility Class I and II genes and increased adhesion of leukocytes to endothelium of blood vessels in the affected tissues. Increased adhesion of leukocytes in these animals was associated with higher expression of VLA4 cell surface markers in the leukocytes. Fibronectin peptide treatment of the knock-out mice blocked inflammation, moderated the body weight and restored homeostatic regulation of immune cell proliferation and inflammation. The mice have anti-DNA and other autoantibodies in the serum. When TGF-beta 1 null bone marrow is transplanted into normal recipients, the recipients develop similar autoantibodies as the knockout mice and most recipients show signs of esophagitis. Collectively, these data indicate that autoimmune mechanisms play a role in the etiology of the inflammatory process in these mice.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02731-09
DMNB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Therapy of Inherited Enzyme Deficiencies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Karlsson, M.D., Ph.D.	Section Chief, M&MG	DMNB	NINDS
Others:	R. Brady, M.D.	Chief	DMNB	NINDS
	S. Klupfel-Stahl	Special Volunteer	DMNB	NINDS
	M. Makoto, M.D., Ph.D.	Special Volunteer	DMNB	NINDS
	R. Schiffmann, M.D.	Visiting Associate	DMNB	NINDS
	M. Amiri, M.S.	Special Volunteer	DMNB	NINDS
	J. Reiser, Ph.D.	Special Volunteer	DMNB	NINDS
	J. A. Medin, Ph.D.	IRTA	DMNB	NINDS

COOPERATING UNITS (if any)

Clinical Hematology Branch, NHLBI (Drs. R. Donahue and C. Dunbar)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.9

PROFESSIONAL:

3.9

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gaucher's disease is an inherited disorder caused by a mutation of the gene for the enzyme glucocerebrosidase. The normal gene for this enzyme has been cloned by several laboratories. We have constructed high-titer, helper-free recombinant retroviruses containing this gene. We have shown that infection of cell lines from normal individuals and patients with Gaucher's disease with this retroviral vector results in increased glucocerebrosidase activity. The glucocerebrosidase gene has been transferred efficiently into progenitor cells and repopulating stem cells of mouse bone marrow, and is expressed at the RNA and protein level in the progeny of CFU-S multipotential progenitor cells following gene transfer. The gene has also been transferred efficiently into murine hematopoietic stem cells that can be used to repopulate secondary transplant recipients. The vector genome can be detected in all hematopoietic lineages and produces human glucocerebrosidase RNA in all hematopoietic tissues tested. High levels of human glucocerebrosidase are generated in hematopoietic tissues. The macrophages of these long-term reconstituted mice produce human glucocerebrosidase levels that are equivalent to the endogenous mouse enzyme levels. Hematopoietic stem cells from rhesus monkeys have been transduced by glucocerebrosidase vector supernatants with a marking efficiency of approximately 1%. The human glucocerebrosidase gene has been introduced into human hematopoietic progenitor cells with a high degree of efficiency. Vector-transduced hematopoietic progenitors from Gaucher patients produce progeny cells with glucocerebrosidase enzyme values similar to those of normal individuals. A clinically acceptable infection protocol has been developed which can be used to correct the enzyme deficiency in hematopoietic cells from Gaucher patients following gene transfer into primitive hematopoietic cells. The first human clinical protocol has started.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02664-11 DMNB		
PERIOD COVERED October 1, 1994 to September 30, 1995				
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical Studies of Neurogenetic Diseases				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)				
PI:	N. Barton, M.D., Ph.D.	Section Chief	DMNB	NINDS
Others:	R. Brady, M.D.	Chief	DMNB	NINDS
	L. Scott, M.D.	Clinical Associate	DMNB	NINDS
	R. Schiffmann, M.D.	Visiting Associate	DMNB	NINDS
	P. Pentchev, Ph.D.	Section Chief	DMNB	NINDS
COOPERATING UNITS (if any) Neuroimaging Branch, NINDS, and Laboratory of Molecular and Cellular Neurobiology, NINDS				
LAB/BRANCH Developmental and Metabolic Neurology Branch				
SECTION Clinical Investigations and Therapeutics				
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892				
TOTAL STAFF YEARS:	2.0	PROFESSIONAL:	1.55	OTHER: 0.45
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>We have identified a novel <u>hereditary demyelinating disorder</u> in children and demonstrated highly specific magnetic resonance spectroscopic alterations that appear unique to this disorder. The latter observation has allowed us to identify a cohort of patients who are undergoing detailed biochemical and molecular biologic investigation. These studies will serve to advance understanding of the hereditary <u>leukodystrophies</u> of unknown etiology. The latter illnesses are not infrequent in occurrence and are particularly devastating for children.</p> <p>We have initiated a series of investigations to define sensitive disease progression markers in patients with <u>Fabry's disease</u>. Patients with this disorder frequently suffer severe neuritic pain early in life and ultimately succumb to cardiac, renal or cerebrovascular disease in the fourth or fifth decade. We have discovered novel MR imaging findings in the CNS of these patients that appear to develop predictably between 35 and 40 years of age. In addition, we have observed previously unreported physiological abnormalities of small myelinated and unmyelinated fibers in the peripheral nervous system. By performing quantitative analyses of the number of free nerve endings in the skin, we have discovered a marked thinning of terminal dendritic arborizations as the disease progresses. Immunohistochemical analyses demonstrate significant regenerative activity in these nerve endings. Further development of these methodologies will permit us to use these parameters to evaluate the effects of enzyme replacement therapy and gene replacement therapy in patients with Fabry's disease.</p>				

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01NS02453-15
DMNB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gaucher's Disease: Biochemical and Clinical Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	N. Barton, M.D., Ph.D.	Section Chief	DMNB, NINDS
Others:	R. O. Brady, M.D.	Chief	DMNB, NINDS
	G. Murray, Ph.D.	Special Volunteer	DMNB, NINDS
	G. Zirzow, B.S.	Biologist	DMNB, NINDS
	K. Oliver, M.S.	Biologist	DMNB, NINDS
	R. Schiffmann, M.D.	Visiting Associate	DMNB, NINDS
	L. Scott, M.D., Ph.D.	Clinical Associate	DMNB, NINDS
	C. Kaneski, B.S.	Biologist	DMNB, NINDS

COOPERATING UNITS (if any)

Massachusetts Gen. Hospital, Dept. of Orthopedic Surgery, Boston, MA: (H. Mankin, D. Rosenthal, S. Doppelt); Children's Hospital, Washington, D. C. (P. Guzzetta)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Clinical Investigations & Therapeutics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.8

PROFESSIONAL:

1.85

OTHER:

1.95

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Extraordinarily gratifying success has been obtained with enzyme replacement therapy in patients with Gaucher's disease. All patients who received macrophage-targeted human placental glucocerebrosidase had significant clinical benefit. The hemoglobin level rose in all patients, and the size of the spleen and liver decreased in all recipients. Long-term treatment has produced reversal of skeleton pathology. Patients who received the enzyme were able to resume activities such as work or school that they had been unable to carry out before enzyme replacement. The U.S. Food and Drug Administration has approved the use of macrophage-targeted glucocerebrosidase as specific therapy for patients with Type 1 Gaucher's disease. The beneficial effect of enzyme replacement in patients with Gaucher's disease has been repeatedly confirmed. The quantity of enzyme that patients require to be maintained in good health is far less than that which is initially necessary to reverse the clinical and pathological manifestations of the disorder. Patients with milder clinical signs of the disorder improve with smaller amounts of enzyme than that required by more severely affected individuals. Recombinantly produced macrophage-targeted glucocerebrosidase has been found to be as effective as the placental enzyme used in the original clinical efficacy trial.

A series of investigations is currently in progress to determine the effects of enzyme replacement therapy on the nervous system in patients with neuronopathic forms of Gaucher's disease. We have demonstrated the delivery of small amounts of enzyme to the nervous system in these patients and are currently analyzing its effects on neurophysiologic parameters and biochemical markers in the cerebrospinal fluid. The outcome of these investigations will significantly influence therapeutic strategies for many inherited metabolic disorders of the nervous system. In addition, we have begun a Phase 1 gene therapy trial in patients with Type 1 (non-neuronopathic) Gaucher's disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 00815-35
DMNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Complex Lipids of Nervous Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	P.G. Pentchev, Ph.D.	Section Chief	DMNB	NINDS
Others:	R.O. Brady, M.D.	Chief	DMNB	NINDS
	J.M. Quirk, M.S.	Biochemist	DMNB	NINDS
	M. Comly, B.S.	Biologist	DMNB	NINDS
	A. Cooney, B.S.	Biologist	DMNB	NINDS
	E.D. Carstea, Ph.D.	Senior Staff	DMNB	NINDS
	P. Chen, Ph.D.	Senior Staff	DMNB	NINDS

COOPERATING UNITS (if any)

LCDB, NIDDK; Lab. Biochem., Fac. Med., Lyon-Sud, France, Depart. of Path., University of North Carolina Medical School, Chapel Hill, N.C.

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Enzymology and Genetics, Molecular and Cellular Pathophysiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	7.9	PROFESSIONAL:	3.0	OTHER:	4.9
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Types C and D Niemann-Pick disease are characterized by abnormal intracellular cholesterol homeostasis. The molecular lesion in these disorders causes: (1) failure to down-regulate LDL receptors on cell membranes; (2) lack of down-regulation of HMGCoA reductase, a key enzyme in cholesterol biosynthesis; and (3) inability to up-regulate acyl cholesterol acyl CoA transferase, the enzyme that catalyzes the esterification of intracellular cholesterol. Tests have been developed and are now widely used in medical practice for the diagnosis of Types C and D Niemann-Pick disease, identification of heterozygotes, and the prenatal diagnosis of these conditions.

We have linked the NP-C mutation to chromosome 18 by positional cloning. We have obtained partial correction of the defective cholesterol metabolism with YACs within the NP-C defined interval on chromosome 18 q11. Identification of the gene will enable us to assess direct DNA diagnosis and initiate protein and gene replacement studies. The Golgi apparatus has been shown to regulate lysosomal cholesterol transport. Characterization of the cholesterol transporter as identified by the NP-C mutation will provide the tools to begin to delineate the molecular mechanisms as well as cellular pathways of intracellular cholesterol transport. Armed with such information, we will study cholesterol processing in normal cells and in pathogenic conditions represented, not only by the NP-C cell, but also by other cholesterol lipidotic states such as atherosclerosis and potentially Alzheimer's disease.

Additional Investigators:

K. Coleman, B.S.
D. Zhang, B.S.Biologist
BiologistDMNB, NINDS
DMNB, NINDS

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02877-03 ERB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Preclinical Evaluation of Novel Anticonvulsant Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael A. Rogawski, M.D., Ph.D. Chief NEXS, ERB, NINDS

Others: Shun-Ichi Yamaguchi, Ph.D. Psychologist NEXS, ERB, NINDS
Sean Donevan, Ph.D. Visiting Fellow NEXS, ERB, NINDS
Tushar Kokate, Ph.D. Visiting Fellow NEXS, ERB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Epilepsy Research Branch

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigation of novel antiepileptic drugs in animal seizure models is carried out as a complement to studies on the interaction of these drugs with ion channels in *in vitro* systems. In the present reporting period, studies were conducted examining the activity of steroid hormones in models of status epilepticus. Endogenous metabolites of certain steroid hormones (neurosteroids) can modulate the excitability of CNS neurons via direct actions on GABA_A receptors. Several structurally related metabolites of progesterone (3 α -hydroxy pregnane-20-ones) and deoxycorticosterone (3 α -hydroxy pregnane-21-diol-20-ones) and their 3 β -epimers were evaluated for protective activity against pilocarpine-, kainic acid- and N-methyl-D-aspartate (NMDA)-induced seizures in mice. Steroids with the 3-hydroxy group in the α -position and 5-H in the α - or β -configurations were highly effective in protecting against pilocarpine (416 mg/kg, s.c.)-induced limbic motor seizures and status epilepticus (ED₅₀ values, 7.018.7 mg/kg, i.p.). The corresponding epimers with the 3-hydroxy group in the β -position were also effective but less potent (ED₅₀ values, 33.863.5, i.p.). Although the neuroactive steroids were considerably less potent than the benzodiazepine clonazepam in protecting against pilocarpine seizures, steroids with the 5 α ,3 α -configuration had comparable or higher protective index values (TD₅₀ for motor impairment/ED₅₀ for seizure protection) than clonazepam, indicating that some neuroactive steroids may have lower relative toxicity. Steroids with the 5 α ,3 α - or 5 β ,3 α - configurations also produced a dose-dependent delay in the onset of limbic seizures induced by kainic acid (32 mg/kg, s.c.), but did not completely protect against the seizures. However, when a second dose of the steroid was administered 1 hr after the first dose, complete protection from the kainic acid-induced limbic seizures and status epilepticus was obtained. The steroids also caused a dose-dependent delay in NMDA (257 mg/kg, s.c.)-induced lethality, but did not completely protect against NMDA seizures or lethality. It was concluded that neuroactive steroids are highly effective in protecting against pilocarpine- and kainic acid-induced seizures and status epilepticus in mice, and may be useful in treating some forms of status epilepticus in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02858-04 ERB															
PERIOD COVERED October 1, 1994 through September 30, 1995																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neuropsychological and Cognitive Studies in Epilepsy																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">William Theodore, M.D.</td> <td style="width: 20%;">Chief, CES</td> <td style="width: 10%;">ERB</td> <td style="width: 10%;">NINDS</td> </tr> <tr> <td>Others:</td> <td>Charles DeCarli M.D.</td> <td>Staff Fellow</td> <td>ERB</td> <td>NINDS</td> </tr> <tr> <td></td> <td>Teresa Blaxton, Ph.D.</td> <td>Staff Fellow</td> <td>ERB</td> <td>NINDS</td> </tr> </table>			PI:	William Theodore, M.D.	Chief, CES	ERB	NINDS	Others:	Charles DeCarli M.D.	Staff Fellow	ERB	NINDS		Teresa Blaxton, Ph.D.	Staff Fellow	ERB	NINDS
PI:	William Theodore, M.D.	Chief, CES	ERB	NINDS													
Others:	Charles DeCarli M.D.	Staff Fellow	ERB	NINDS													
	Teresa Blaxton, Ph.D.	Staff Fellow	ERB	NINDS													
COOPERATING UNITS (if any)																	
LAB/BRANCH Epilepsy Research Branch, CNP, DIR																	
SECTION Clinical Epilepsy Section																	
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892																	
TOTAL STAFF YEARS: 3.1	PROFESSIONAL: 2.5	OTHER: 0.6															
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews								
<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither															
<input type="checkbox"/> (a1) Minors																	
<input type="checkbox"/> (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have been performing <u>imaging studies of language organization</u> in normal controls and patients with epilepsy. Using positron emission tomography (PET), activation of cerebral blood flow (CBF) associated with word and object recognition, auditory comprehension, and phoneme, word, and sentence production are localized in the brain. Studies of these functions in normals form the basis for evaluating the <u>effect of seizure disorders on cognitive processes</u> subserved by temporal lobe and other cerebral structures. In studies of memory, deactivation of cerebral blood flow during retrieval reflects the effects of earlier encoding. The deactivated regions are those which are engaged in the initial processing of stimuli. During a visual design recognition task, we found deactivation of right primary visual cortex. During an auditory recognition task, there was a relative CBF decrease in bilateral superior and middle temporal regions. We also evaluated a classical learning paradigm using eyeblink conditioning. We found learning specific increases in CBF in regions previously implicated in animal studies, including cerebellum, basal ganglia, frontal cortex, and hippocampus. In patients with left temporal foci, PET showed functional reorganization of language processing. Data from subdural stimulation, PET, and magnetic resonance imaging (MRI) are integrated using digital image processing techniques. The combined stimulation and PET data allow us to study the relationship between activation and disruption of cognitive activity, and to form more accurate concepts of the organization of cerebral function. These studies will elucidate the function of regions such as the basal temporal language area, which are of clinical importance when surgery for uncontrolled seizures is planned. Digital signal processing techniques are used to align PET, CT, MRI, and subdural electrode positions. We found a high concordance between PET-CBF and subdural stimulation mapping using a number of different functional tests. This result shows the practicality of noninvasive preoperative functional brain mapping, and also demonstrates the close correlation of disruption and activation studies. We have found significant involvement of the basal temporal language area, which may explain unexpected postoperative deficits, in PET activation studies. During subdural mapping, stimulation of the basal temporal region disrupted implicit memory priming. Using <u>fMRI in children</u>, we found increased signal in left inferior frontal and temporal regions. This noninvasive technique can be used to lateralize speech. </p>																	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02772-08 ERB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Uncompetitive NMDA Antagonists as Anticonvulsants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Rogawski, M.D., Ph.D.	Chief	NEXS, ERB, NINDS
Others:	Shun-Ichi Yamaguchi, Ph.D.	Psychologist	NEXS, ERB, NINDS
	Tushar Kokate, Ph.D.	Visiting Fellow	NEXS, ERB, NINDS
	Swaminathan Subramaniam, M.D., Ph.D.	Visiting Asst.	NEXS, ERB, NINDS
	Duan-chan Uyakul, Ph.D	LAC, NIDDK	
	Lewis K. Pannell, Ph.D.	LAC, NIDDK	

COOPERATING UNITS (if any)

Department of Pharmacology, University of Massachusetts Medical Center; Neurogen Corporation, Branford, CT

LAB/BRANCH

Epilepsy Research Branch

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	1.4	PROFESSIONAL:	1.4	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

ADCI (5-aminocarbonyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine), a low- -affinity uncompetitive NMDA antagonist, is a broad-spectrum anticonvulsant with a favorable side effect profile. However, the drug's clinical utility will be dependent upon its ability to maintain efficacy when it is administered to patients on a chronic basis. Therefore, we sought to determine if tolerance develops to the anticonvulsant activity of chronically administered ADCI using the mouse maximal electroshock (MES) test to assess seizure protection. In studies with chronic ADCI administration, it was observed that tolerance does occur, but that this is due to pharmacokinetic factors (enhanced first-pass metabolism) and does not result from a reduction in anticonvulsant efficacy. The mechanism of the low-toxicity of ADCI is not well understood, but could relate, in part, to selective actions of the drug on distinct NMDA receptor subtypes. Preliminary studies indicated that ADCI produces a more potent block of NMDA receptors containing the alternatively spliced NR1₁₁ subunit (which includes a 21-amino acid N-terminal insert coded by exon 5 of the NR1 gene) than NR1₀₁₁ (which lacks this insert).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02732-09 ERB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological Studies of Ion Channels in Cultured Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael A. Rogawski, M.D., Ph.D. Chief NEXS, ERB, NINDS

Others: Sean Donevan, Ph.D. Visiting Associate NEXS, ERB, NINDS
Swaminathan Subramaniam, M.D., Ph.D. Visiting Associate NEXS, ERB, NINDS
Jong Rho, M.D. Medical Staff Fellow NEXS, ERB, NINDS
Karen Wayns, B.S. Biologist NEXS, ERB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Epilepsy Research Branch

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.4

PROFESSIONAL:

3.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Whole-cell voltage-clamp and single channel recording techniques were used to study drug interactions with N-methyl-D-aspartate (NMDA) and non-NMDA receptor coupled cation and γ -aminobutyric acid_A (GABA_A) receptor-coupled Cl channels in cultured hippocampal neurons. The aim of this work was to explore new strategies for the rational development of antiepileptic drugs based upon their interaction with neuronal ion channel systems. Work was focused in the following areas: (i) pharmacology of Ca²⁺-permeable, inwardly rectifying AMPA receptors, (ii) mechanism of inward rectification of Ca²⁺-permeable AMPA receptors; (iii) mechanism of action of felbamate and the related dicarbamate meprobamate, (iv) effects of the polyamine toxins argiotoxin 636 (ARG 636) and philanthotoxin 434 (PHTX 434) on NMDA receptors; and (v) studies on the interaction of the anticonvulsant remacemide and its des-glycine metabolite with NMDA receptors. AMPA receptors lacking the GluR2 subunit have an inwardly rectifying current-voltage relationship and are permeable to Ca²⁺; a subpopulation of cultured hippocampal neurons selectively express these receptors. The polyamine toxins PHTX 343 and ARG 636 were found to selectively block Ca²⁺-permeable AMPA receptors in these neurons, but to have minimal effects on neurons expressing Ca²⁺-impermeable AMPA receptors. Support was obtained for the hypothesis that inward rectification of Ca²⁺-permeable AMPA receptors occurs as a result of voltage-dependent channel block by internal polyamines. Felbamate, a newly approved antiepileptic agent, and the related dicarbamate meprobamate were found to inhibit NMDA receptors by a channel blocking action and also possibly by distinct effects on channel gating. It has been suggested that the NMDA receptor blocking activity of felbamate may result from competitive antagonism at the glycine recognition site of the NMDA receptor. Several observations failed to support such a glycine site interaction. PHTX 343 and ARG 636 were found to inhibit NMDA receptors via open channel block and competitive antagonism at the NMDA recognition site. In addition, ARG 636 exerted a polyamine-like facilitation of NMDA receptor currents. The interaction of the novel anticonvulsant remacemide and its des-glycine metabolite with NMDA receptors was examined in whole cell voltage-clamp recordings from cultured rat hippocampal neurons and in binding studies with [³H]dizocilpine in rat forebrain membranes. The metabolite was found to be a potent, stereoselective open channel blocker whereas remacemide itself was a weak NMDA receptor antagonist which acted as an allosteric antagonist and also, like the metabolite, as a channel blocker. Overall, the functional NMDA receptor blocking activity of remacemide in vivo is likely to be mediated predominantly by the des-glycine metabolite.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02318-18 ERB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of Antiepileptic Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William H. Theodore, M.D.	Chief, CES	ERB	NINDS
Others:	Charles DeCarli, M.D.	Medical Officer	ERB	NINDS
	Andy Dean, M.D.	Clinical Associate (SF)	ERB	NINDS
	Pat Reeves	Technician	ERB	NINDS
	Hugh Malek	Technician	ERB	NINDS
	Kathy Kelley	Technician	ERB	NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	3.2	PROFESSIONAL:	2.4	OTHER:	0.8
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input checked="" type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies using positron emission tomography (PET) showed that drugs which interact with the GABAergic system may be more likely to reduce CMRglc than those which do not. We found that valproic acid depressed CBF in the thalamus to a greater degree than in other regions. This effect may be related to the drug's efficacy against primary generalized seizures. In a double-blind placebo-controlled parallel design trial, we are investigating the effect of gamma-vinyl-GABA (GVG) an experimental antiepileptic drug, on cerebral glucose metabolism (CMRglc), blood flow (CBF), CSF GABA levels, and seizure frequency, in patients with uncontrolled complex partial seizures (CPS). Vigabatrin raises CSF GABA, and this effect may be related to seizure control. We are particularly interested in the possibility of regional reductions in LCMRglc or CBF, which predict efficacy or toxicity, including psychiatric disorders. Vigabatrin causes intramyelinic edema in laboratory animals, which has not been found on human MRI or evoked potential studies. PET may be more sensitive than these modalities to subtle abnormalities. Patients who have persistent seizures on carbamazepine therapy (CBZ) will be entered into the study. After initial evaluation to confirm seizure type, establish therapeutic CBZ levels, and exclude patients with evidence of systemic or progressive neurological disease, a baseline PET scan, lumbar puncture, MRI, evoked potential and neuropsychological test battery will be performed. Patients then will be randomized to placebo or vigabatrin in addition to continuous CBZ therapy. Vigabatrin / placebo will be titrated upwards to 50 mg / kg over 3 weeks. After two months the PET scan and other tests will be repeated. The primary outcome measure of the study will be the drug effect on CBF and LCMRglc.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02236-20 ERB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diagnostic and Therapeutic Reevaluation of Patients With Intractable Epilepsy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William H. Theodore, M.D.	Chief, CES	ERB	NINDS
Others:	Irene Von Albertini, M.D.	Clinical Associate	ERB	NINDS
	Charles DeCarli, M.D.	Medical Officer	ERB	NINDS
	Andy Dean, M.D.	Clinical Associate (SF)	ERB	NINDS
	Pat Reeves	Technician	ERB	NINDS
	Hugh Malek	Technician	ERB	NINDS
	Kathy Kelley	Technician	ERB	NINDS

COOPERATING UNITS (if any)

EEG Laboratory, Office of The Clinical Director, NINDS

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.8

PROFESSIONAL:

2.2

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

☒

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☒

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients are selected after video-EEG monitoring to determine seizure type and focus localization. Some patients have chronic subdural recording. PET and MRI are used to study cerebral blood flow (CBF) and glucose metabolism (FDG-PET) in patients with uncontrolled complex partial seizure disorders to investigate the relation of substrate supply to utilization, and the role of imaging in presurgical localization of epileptic foci. Pre- and postoperative scans are compared to assess the effect of resection on regions distant from the epileptic focus. Both LCMRglc and CBF were significantly depressed ipsilateral to the epileptic focus. However, LCMRglc was depressed by a mean of 11.2%, and CBF by only 3.2% in the lateral temporal cortex. In the mesial cortex, LCMRglc was depressed by 11.1% and CBF by 6.1%. The ratio of LCMRglc to CBF was significantly depressed ipsilateral to the focus. Using standardized criteria, blind raters found that 80% had depressed LCMRglc but only 50% of patients depressed CBF on either $H_2^{15}O$ -PET or $99m Tc$ -SPECT. Compared to EEG foci localized by ictal video-EEG telemetry, blood flow studies were falsely lateralizing in about 10% of patients. Using image coregistration, we compared FDG-PET to hippocampal formation volume. In this group of patients, 89% had regional hypometabolism, 61% focal T2 increases, 50% absolute HF atrophy ipsilateral to the focus. 55% had abnormal L/R HF ratios, with a smaller HF ipsilateral to the EEG focus. All patients with abnormal volumetric MRI had abnormal PET. There was a significant correlation between HF volume and inferior mesial and lateral temporal hypometabolism, suggesting that depressed LCMRglc may reflect HF atrophy. PET is more sensitive than MRI volumetry in identifying the ictal focus, but does not provide additional information when HF atrophy is present. In contrast to adults, children (mean age 14.7 years) in our series were more likely to have hypometabolism (69%) than abnormal MRI (25%). Depression among our patients, measured by the Beck Depression Inventory, was associated with reduced bilateral inferior frontal metabolism compared to both normal controls and non depressed patients with epilepsy. We are particularly interested in the value of PET scanning when surface EEG is nonlocalizing. We are analyzing data from subcortical regions with MRI based image analysis, and studying post-resection patterns of LCMRglc and CBF, to determine if there are any changes outside the resected region, which are associated with success or failure.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02826-05 ETB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Regulation of Transmitter Receptor Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Maral Mouradian, M.D.	Chief, Genetic Pharmacology	ETB/NINDS
Others:	S. Yajima, Ph.D.	Visiting Fellow	GPU, ETB
	S-H. Lee, Ph.D.	Visiting Fellow	GPU, ETB
	J. Bishop, M.S.	Biologist	GPU, ETB
	W. Wang, M.D.	Special Volunteer, Georgetown U & GPU, ETB	
	P.A. Jose, M.D., Ph.D.	Guest Researcher	GPU, ETB

COOPERATING UNITS (if any)

Dept. Physiology, USUHS; Dept. Pediatrics, Georgetown Univ. Med. Center; Molecular Neurobiology, Dept. Neurology, Univ. Tokyo

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Genetic Pharmacology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.6

PROFESSIONAL:

3.6

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long-term goal of this project is the development of genetic approaches to treat neurodegenerative diseases. One of the strategies pursued is the discovery of small molecules or oligodeoxynucleotides that alter the expression of relevant endogenous genes within the brain. The genes investigated are those encoding two neurotrophic factors brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) and the two dopamine receptor subtypes expressed in the striatum, D_{1A} and D₂.

1. We found that mRNA levels of both BDNF and GDNF genes are markedly enhanced in a glioma cell line by increased intracellular calcium levels. This observation provides an explanation for the well-known dramatic increase in BDNF expression with ischemic insult, seizures and trauma all of which result in the release of glutamate and influx of CA²⁺. The five alternate promoters in the BDNF gene responded to different degrees to the calcium ionophore.

2. We had previously characterized the structure of the human D_{1A} dopamine receptor gene. We now discovered that its transcriptional control is quite complex with regulatory sequences and DNA/protein binding sites along the approximately 900 bp long 5' untranslated region. We also found that POU family of transcription factors differentially activate this gene.

3. The D₂ dopamine receptor is traditionally recognized to be the main mediator of the motor and endocrine effects of dopamine. We had previously found a strong negative modulatory sequence in this gene which binds to a 130 kDa protein and Sp1. During FY95, we cloned this unknown protein as well as four additional transcription factors all belonging to the zinc finger family of proteins.

4. The D₃ dopamine receptor is expressed at low levels in the motor striatum which might be important for relaying dopaminergic messages to the output regions of the basal ganglia. We found that chronic dopaminergic blockade with several antipsychotic drugs increases D₃ mRNA levels in olfactory tubercle and nucleus accumbens of rats but not in motor striatum. Thus, the extrapyramidal syndrome of chronic neuroleptic therapy is not due to D₃ receptor upregulation in the striatum. Consequently, antagonists selective of the D₃ subtype may be effective antipsychotics without the adverse motor effects.

(This project is partially supported by the Gift Fund, CAN 58328742.)

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02263-19 ETB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Pharmacological Studies of Dopamine Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: David R. Sibley, Ph.D. Chief MNS/ETB/NINDS

Others: Jean E. Lachowicz, Ph.D., Staff Fellow; Tom R. Hollon, Ph.D., IRTA Fellow; Dong-Jiang, Ph.D., IRTA Fellow; Steven I. Max, Ph.D., IRTA Fellow; Loyd H. Burgess, Ph.D., IRTA Fellow; Chun Mak, Ph.D., IRTA Fellow; John A. Schetz, Ph.D., IRTA Fellow; Bitu Nakhai, Ph.D., Visiting Fellow; Ana Ventura, Ph.D., Guest Researcher; Naoko Ozaki, Ph.D., Special Volunteer

COOPERATING UNITS

Lab of Mammalian Genes & Dev, NICHD; Neurosci Dep., Chicago Med School; Pharm Dep., Texas Tech Univ; Psychiat Dep., Seattle VA MC; Psychiat Dep., Case Western Reserve U; Mental Retard Ctr, UCLA

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Molecular Neuropharmacology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

7.1

PROFESSIONAL:

7.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long-term goal of this project is the characterization of neurotransmitter receptor-mediated information transduction, and its regulation, across neuronal membranes. The primary, but not exclusive, model systems under investigation are those for dopamine receptors. In order to characterize dopamine and related receptors at the biochemical and molecular levels and study their regulation, there are two major interrelated lines of research which are ongoing: (1) investigation of the cell biology, function and regulation of the receptors at the protein level; and (2) the molecular cloning of the receptor cDNAs/genes and investigation of receptor structure and regulation in normal and pathophysiological states.

(1) Cell Biology and Regulation of Dopamine Receptors. Characterization of the functional and regulatory properties of D-1 and D-2 dopamine receptors in various cDNA-transfected cell lines was continued. The role of cAMP in agonist-induced regulation of D-1 and D-2 receptors is being investigated using cell lines deficient in the cAMP-dependent protein kinase as well as using site-directed mutagenesis techniques to alter potential phosphorylation sites in the receptor proteins. Chimeric D-1/D-2 receptors have been constructed to further investigate regulatory mechanisms of these receptors. D-2, D-3 and D-4 receptors were transfected into the mesencephalic cell line, MES 23.5. D-2 and D-3 receptors were found to increase K⁺ currents whereas D-4 receptors decreased this response. Agonist-induced desensitization of the 5-HT-6 serotonin receptor was characterized. Interestingly, this receptor exhibited a desensitization response in the absence of down-regulation.

(2) Molecular Biology of Dopamine Receptors. Work continued on cloning of a third "D-1 like" receptor which apparently is linked to the stimulation of phosphatidylinositol turnover and mobilization of calcium. Transgenic "knock-out" mice lacking a functional D-1 receptor were further characterized and transgenic mice lacking the D-3 receptors were produced. Other transgenic mice lacking the D-5 receptors are in production. Chimeric D-2/D-3 and D-2/D-4 dopamine receptors were constructed and expressed for characterization. These results indicated that multiple domains in the D-2 receptor are important for coupling to adenylyl cyclase and that transmembrane region III is an important structural determinant for D-4 pharmacology. The D-4 receptor pharmacology was further found not to be predictive of atypical antipsychotic efficacy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02265-19 ETB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Pharmacology, Biochemistry and Physiology of Central Neurotransmitters		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> PI: Thomas N. Chase, M.D. Chief ETB/NINDS Others: Jeff Anderson, PhD, IRTA Fellow; Robert Boldry, PhD, IRTA Fellow; Stella Papa, M.D., Visiting Fellow; Leo Verhagen-Metman, M.D. Visiting Associate; Pierre Blanchet, M.D., Special Volunteer		
COOPERATING UNITS <small>(if any)</small> The Upjohn Company, Kalamazoo, MI; Nagoyo University; PET Center, Brussels; NIMH		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Clinical Pharmacology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 4.41	PROFESSIONAL: 4.41	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <div style="margin-left: 20px;"> <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>Studies of the altered motor responses to <u>levodopa</u> occurring in advanced <u>Parkinsonian patients</u> revealed that the latency-to-peak motor response correlated closely with symptom duration and severity. The results suggest that compensatory presynaptic mechanisms, especially accelerated dopamine turnover and release, contribute to the absence of Parkinsonian symptoms until most nigrostriatal dopaminergic neurons have degenerated. In a related laboratory investigation, levodopa treatment of Parkinsonian rats with stable destruction of more than 95% of their dopaminergic neurons leads to a progressive shortening in the duration of levodopa's motor effects. This response alteration, resembling the wearing-off phenomena that develop in Parkinsonian patients, did not occur in less severely lesioned rats, suggesting that postsynaptic changes loss of dopamine neurons must exceed a relatively high threshold before levodopa treatment produces changes, evidently at the postsynaptic level, that favor the rapid appearance of wearing-off fluctuations. A subsequent clinical study confirmed that, contrary to long-held beliefs, postjunctional alterations, possibly involving relatively plastic striatal dopaminergic systems, also account for most of the shortening in the duration of levodopa action that underlie wearing-off fluctuations in parkinsonian patients. Previous studies by the Section demonstrated the ability of the <u>glutamate</u> system to alter motor responses to dopaminergic stimulation. In addition, we found that repeated levodopa administration down-regulated D1 dopamine receptor mediated responses, up-regulated those mediated by D2 receptors, and progressively reduced the duration of the motor response to levodopa. Acute co-treatment with the noncompetitive NMDA antagonist MK-801 completely reversed all these changes, suggesting that NMDA receptor-mediated mechanisms contribute to the response changes associated with chronic levodopa treatment and that NMDA antagonists act to reverse the motor response complications attending long-term levodopa therapy. Since degeneration of cortical glutamatergic projections is a consistent finding in Alzheimer's disease, we evaluated the possibility that glutamate system stimulation might confer symptomatic benefit. Cycloserine, an indirect agonist at certain (NMDA) glutamate receptors, had no consistent effect on neuropsychological outcome measures, suggesting that short-term potentiation of NMDA-mediated glutamatergic transmission may not prove useful in the symptomatic treatment of this disorder.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02139-21 ETB

PERIOD COVERED

October 1, 1994 through September 31, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology and Physiology of the Substantia Nigra and Basal Ganglia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Judith R. Walters Chief, Neurophysiological Pharmacology Section ETB/ NINDS

Others:	Debra Bergstrom	Pharmacologist-	ETB/NINDS
	Michael Twery	Senior Staff Fellow	ETB/NINDS
	Kai-Xing Huang	Special Volunteer	ETB/NINDS
	Lisa Thompson	Staff Fellow	ETB/NINDS
	Deborah Kreiss	Staff Fellow	NIGMS

COOPERATING UNITS (if any)

Clinical Pharmacology Section, Experimental Therapeutics Branch

LAB/BRANCH

Experimental Therapeutics Branch, CNP

SECTION

Neurophysiological Pharmacology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.45

PROFESSIONAL:

5.45

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	<input type="checkbox"/>	(b) Human tissues	<input checked="" type="checkbox"/>	(c) Neither
<input type="checkbox"/>	(a1) Minors				
<input type="checkbox"/>	(a2) Interviews				

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to elucidate the function of specific neuronal systems in regulating information processing in the basal ganglia in order to identify mechanisms which could be manipulated to prevent, correct and/or compensate for dysfunction involving these systems. In FY95, focus has been on processes underlying the effects of dopamine receptor subtypes on basal ganglia output.

(1) Subthalamic nucleus: *In vivo* extracellular single unit recordings have shown, unexpectedly, that locally as well as systemically administered dopamine D1 agonists exert excitatory effects on the activity of subthalamic neurons. Systemic effects are blocked by local infusion with a D1 antagonist. D2/D3 agonists induce less change in subthalamic activity although tonic endogenous tone at D2/D3 receptors appears required for the D1-mediated effects. Autoradiographic techniques have demonstrated D2/D3 receptors in the subthalamic nucleus and D1 receptors on the ventral border adjacent to the cerebral peduncle; presumably located on terminals of excitatory cortical afferents. Thus, in addition to acting on striatopallidal and striatonigral neurons, dopaminergic agents also may exert functionally significant effects on basal ganglia output through actions on cortical inputs to the subthalamic nucleus. In animal models of Parkinson's disease, tonic activity in the subthalamic nucleus is enhanced and the responses to dopamine agonists is markedly altered. Nonselective dopamine agonist induces decreases in subthalamic activity in the lesioned rats as compared to increases in normal animals. Mechanisms are being investigated and may be relevant to the efficacy of pallidotomies in the treatment of Parkinson's disease.

(2) Striatum: AP-1 transcription factor binding in rat striatal nuclear protein extract is enhanced in a synergistic manner after combined systemic administration of D1 and D2/D3 agonists. These results demonstrate that D1 and D2/D3 receptor-mediated synergistic interactions induce changes in striatal gene expression as well as neuronal activity but do not indicate where the receptor subtypes are localized. Infusing selective D1 and D2 agonists locally into the striatum in 6-OHDA-lesioned rats produces changes in Fos protein in the striatum; however, this does not coincide with alterations in basal ganglia output as measured by firing rates in the SNpr. Results indicate that D1 and D2 receptors in the striatum as well as D1 receptors at extrastriatal sites are necessary for mediating the changes in basal ganglia output associated with D1 and D2/D3 dopamine receptor stimulation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02901-02

MNB*

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of the Autosomal Dominant Cerebellar Ataxias

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Joseph J. Higgins, M.D.	Senior Clinical Investigator	CNU, MNB, NINDS
Others:	Lev Goldfarb, Ph.D.	Chief, Clinical Neurogenetics Unit	MNB, NINDS
	Irwin Kopin, M.D.	Chief, Clinical Neuroscience Branch	DIR, NINDS
	Linda E. Nee, M.S.W.	Social Science Analyst	CNU, MNB, NINDS
	Jordan Grafman, Ph.D.	Chief, Cognitive Neuroscience Section	MNB, NINDS
	Susumu Sato, M.D.	Chief, Electroencephalography Section	OCD, NINDS

COOPERATING UNITS (if any)

E. J. Fitzgibbon, M.D., N. Patronas, M.D., C.C; A. Pikus, M.A., NIDCD; M. Polymeropoulos, M.D., NCHGR

LAB/BRANCH

Medical Neurology Branch

SECTION

Clinical Neurogenetics Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Sixty-four affected patients with autosomal dominant cerebellar ataxia from 28 families with at least three consecutive generations were recruited from 496 individuals referred by the Chesapeake Chapter of the National Ataxia Foundation located in Montgomery County, Maryland. Six individuals from 3 families had the SCA1 mutation (11%), 22 individuals from 8 families had the SCA3 mutation (29%), and 36 individuals from 17 families had neither mutation (36%). The point prevalence in Montgomery County, Maryland is estimated to be 2 per 100,000 persons. The number of CAG repeats on chromosome 14q was highly polymorphic with 16 different normal alleles ranging between 14 and 33 repeat units. The lower expansion of the CAG repeat in all affected individuals ranged between 67 and 83 repeats. Several larger alleles containing greater than 200 repeats were visualized on VISIGEL™. The identity of the higher alleles was confirmed as containing (CAG)_n repeats by Southern blot hybridization with a (CAG)_n repeat probe. Sequencing of the CAG repeat region in four expanded alleles demonstrated the presence of a polymorphism at codon 300. An unsteady gait was reported as the first or second symptom in 18 of 19 affected individuals followed by visual disturbances in 9 patients. Cranial nerve findings, tone, reflexes, peripheral signs and muscle strength varied with the severity of the illness. Dystonia and Parkinsonian signs were notably absent in all patients studied. A comparison of 30 quantitative MRI images in affected American individuals with SCA3 and 21 quantitative MRI images in affected American individuals with SCA1 compared with normal controls, indicated a predominant spinopontine atrophy in SCA3 and a severe cerebellar atrophy in SCA1. These molecular, radiologic and clinical findings suggest that SCA3 is allelic to Machado Joseph disease. Decreased levels of CSF HVA, with normal levels of CSF 5-HIAA were found in our patients with SCA1 and SCA3. The CSF ratio of HVA/5-HIAA was lower in patients with SCA3 when compared to patients with SCA1. These findings may explain the lack of a therapeutic response to pharmacological agents that alter serotonin metabolism in patients with ADA. The lack of Parkinsonian signs in our patients with SCA1 and SCA3 implies that striatal dopamine deficiency is not marked early in the course of ataxia but the direct relationship between CSF HVA levels and the midsagittal pontine area on MRI indicates that depletion of dopaminergic neurons does play a role in the pathogenesis of these genetic disorders.

*Formerly Clinical Neuroscience Branch.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02902-02
MNB*

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Biotin-Responsiveness in Familial Pyruvate Carboxylase Deficiency

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Joseph Higgins, M.D. Senior Clinical Investigator CNU, MNB, NINDS

Others: Lev Goldfarb, M.D., Ph.D. Chief, Clinical Neurogenetics Unit MNB, NINDS
Jordan Grafman, Ph.D. Chief, Cognitive Neuroscience Section MNB, NINDS
Linda E. Nee, M.S.W. Social Science Analyst CNU, MNB, NINDS
Susumu Sato, M.D. Chief, Electroencephalography Section OCD, NINDS

COOPERATING UNITS (if any)

Anita Pikus, M.A., NOB, NIDCD; Michalis Polymeropoulos, M.D., NICHGR

LAB/BRANCH

Medical Neurology Branch

SECTION

Clinical Neurogenetics Unit

INSTITUTE AND LOCATION

NINDS, NIH Bethesda, MD 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to document genotypic differences and biotin-responsiveness in family pedigrees with pyruvate carboxylase deficiency. Brainstem and diffuse subcortical white matter changes are found on MRI in several members of this kindred. Improvement in myelination is found on serial MRI after one child received biotin therapy. The pathophysiologic basis underlying these myelin changes may involve PC's role in providing citrate and cytosolic acetyl Co-A as sources for fatty acid synthesis. It is not clear whether the improved myelination in this patient is a consequence of early biotin treatment or simply reflects the natural course of this disease. His parents' fibroblast PC enzyme activity (55-65% of the mean of controls) was consistent with that predicted for obligate heterozygotes. There was a small increase in PC activity in the parent's lymphocyte PC activity. The genetic sequence of the biotin binding domains in all family members appear normal. Studies are in progress to define the mutations in the PC gene responsible for this disorder.

* Formerly Clinical Neuroscience Branch.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02903-02
MNB*

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Use of Attenuated Hepatitis A as a Vector for Neurogene Transfer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Joseph Higgins, M.D.

Senior Clinical Investigator

CNU, MNB, NINDS

Others: Susan A. Zullo, Ph.D.

Pre-IRTA

CNU, MNB, NINDS

COOPERATING UNITS (if any)

G. Kaplan, Ph.D., CBER

LAB/BRANCH

Medical Neurology Branch

SECTION

Clinical Neurogenetics Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

0.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatitis A virus (HAV) exclusively infects the gastrointestinal tract of primates and does not naturally infect the central nervous system. To test HAV's ability to replicate in neural tissue, cultured human fetal brain tissue (HFBT) was infected with HAV/7. *In vitro* cytopathic effects were not seen after infection. Semiquantitative slot blot analyses, using RNA dilutions of 1:10 and 1:100, documented HAV/7 replication in HFBT up to 19 days after the initial infection. Immunofluorescent staining using a monoclonal antibody against the viral capsid demonstrated the presence of HAV/7 and counterstaining with a monoclonal neurofilament (68kd) antibody identified HAV/7 in the cytoplasm of neurons. To test the safety, efficacy and *in vivo* ability of HAV/7 to replicate in the central nervous system, 5×10^6 TCID₅₀/ml was directly inoculated into the thalami and lumbar spinal cords of four *Mucaca mulatta* monkeys. The animals were euthanized at 1 hour, 1 week, 2 weeks and 4 weeks after inoculation. RT-PCR was used to detect the presence of small quantities of the negative strand RNA replication intermediate (RI). To overcome the high degree of secondary structure present in the highly conserved 5' NCR of the HAV RNA, a thermostable reverse transcriptase (rTth DNA polymerase) permitted transcription to proceed at a higher temperature. The PCR products were hybridized with either a ³²P-labeled full length HAV cDNA (10kb) or an overlapping PstI HAV cDNA (687 bp) fragment. Both probes identified a specific 251-bp fragment. RT-*in situ* PCR identified the RI in the cytoplasm of neurons in widespread areas of the brain and spinal cord in tissue samples from these monkeys. Histological evaluation of the adjacent spinal cord and brain areas did not show gliosis, vacuolization or an inflammatory response. The 5' NCR region of HAV/7 was cloned into the plasmid pALTER-1 preparation for oligonucleotide-directed mutagenesis downstream from the IRES. Two unique restriction enzyme sites (9 base pairs) were cloned at nt 776 by annealing a 50 base pair mutagenic oligonucleotide to a double-stranded DNA template. The CAT gene was inserted in-frame at this site and transcription was driven by the plasmid T7 promoter. Fetal rhesus monkey kidney cells were lipofected with the DOTAP reagent. CAT activity was measured by ELISA at days 7, 12, 16, 19 and 26 post-transfection. Low levels of CAT (20-30 pg/ug protein) expression were detected at day 16 post-transfection. These studies suggest that HAV/7 can act as a vector for viral-mediated gene transfer.

* Formerly Clinical Neuroscience Branch.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02909-02 MNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The use of Modern Neuroscience Techniques to Perform Brain-Behavior Mapping

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jordan Grafman, Ph.D.	Chief, CNS, MNB, NINDS
Others:	S. Flitman, M.D.	Clinical Associate, (C.O.), CNS, MNB, NINDS
	T. Rickard, Ph.D.	IRTA Fellow, CNS, MNB, NINDS
	M. Stark, Ph.D.	IRTA Fellow, CNS, MNB, NINDS
	D. Kimberg, Ph.D.	IRTA Fellow, CNS, MNB, NINDS
	I. Litvan, Ph.D.	IRTA Fellow, NEB, NINDS
	*	

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Cognitive Neuroscience Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	4.05	PROFESSIONAL:	4.05	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current neuroscience techniques used in the Cognitive Neuroscience Section include positron emission tomography (PET), functional magnetic resonance imaging (fMRI), rapid-rate transcranial magnetic imaging (rTMS), event-related brain potentials (ERPs), and pharmacologic challenges (PC). Primarily within-group designs are used although both fMRI and rTMS can be used with single-cases and our research is proceeding in this direction. Our PET program is focusing on developing methods to reliably activate various locations within the human prefrontal cortex. In this regard, we are using tasks that require subjects to plan, to develop thematic knowledge, to put themselves in someone else's point-of-view and to mentally execute a set of activities. The fMRI technique is currently being used to study basic cognitive processes such as word production, mental calculation, and selective attention. rTMS is being used to map cortical functions during activity and learning, to interfere with ongoing cognitive processing, and to facilitate cognitive processing. The ERP recordings are being used in order to better identify the components of verbal and nonverbal working memory in normal subjects and in clinical populations. We have judiciously used PC in order to examine the effects of anticholinergic medication on autobiographical memory and selective attention.

*Continued:

M. Hallett, M.D.	Chief, MNB, NINDS
N. Sadato, M.D.	HMCS, MNB, NINDS
A. Pascual-Leone, M.D.	HMCS, MNB, NINDS
P. Pietrini, M.D.	LNS, NIA
E. Wasserman, M.D.	HMCS, MNB, NINDS
D. Ruchkin, Ph.D.	Dept. of Physiology, Univ. of Maryland

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 N5 02876-03
MNB*

PERIOD COVERED

October 1, 1994, through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genotypic-Phenotypic Correlations in Hereditary Movement Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Lev G. Goldfarb, M.D., Ph.D.	Chief	CNU, MNB, NINDS
Other	Olavo Vasconcelos, M.D.	Visiting Fellow	CNU, MNB, NINDS
	Tinatin Chabrashvili, M.D.	Visiting Fellow	CNU, MNB, NINDS
	Mark Dubnick, P.h.D.	Senior Staff Fellow	NS, BNP, NINDS
	James Nagle	Biologist	NS, BNP, NINDS
	Joab Chapman, Ph.D.	Special Volunteer	CNU, MNB, NINDS
	Shira Chapman, M.Sc.	Special Volunteers	CNU, CNP, NINDS

COOPERATING UNITS (if any)

C. OToro, M.D., S. Massaquoi, M.D., L. E. Nee, M.S.W., K. Sivakumar, M.D., M. Dalakas, M.D., MBN, L. Cervenakova, M.D., D. C. Gajdusek, M.D., LCNSS, M. Polymeropoulos, M.D., NCHGR, V.P. Alexeev, M.D., F. Platonov, M.D., S. Kononova, Ph.D., Yakutsk

LAB/BRANCH

Medical Neurology Branch, CNP

SECTION

Clinical Neurogenetics Unit,

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke, Park Building

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

1.75

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Siberian kindred with autosomal dominant cerebellar ataxia type-1 includes 1484 individuals, 225 affected and 656 at risk. The expanded allele in each of 78 examined patients contained a stretch of 39 to 72 uninterrupted CAG repeats in the SCA1 gene on chromosome 6. The normal allele had 25 to 37 trinucleotide repeats. The number of CAG repeats inversely correlated with the age of disease onset and the duration of illness. Progressive cerebellar deficiency was expressed in all patients, independently of the repeat number; the associated symptoms, diffuse skeletal muscle atrophy, dysphagia, tongue atrophy with fasciculation and ophthalmoparesis, had a significantly higher prevalence among patients with the number of CAG repeats 52 and over. Two symptomatic individuals had CAG repeat expansion on both chromosomes. A CAG trinucleotide repeat expansion was also identified in the coding region of the Machado-Joseph disease gene on chr 14q in nine American families of Portuguese, German, Irish and Dutch-African ancestry. All 25 affected members were heterozygous for an expanded allele containing 67 to 83 CAG repeats whereas the normal allele had 6 to 33 repeats. We have identified two novel mutations in the phosphofructokinase muscle subunit gene associated with glycogenosis type VII (Tarui's disease) in three patients from an Ashkenazi Jewish family: a nonsense mutation at codon 95 and 252-nucleotide insertion totally homologous to the structure of the 10th intron. The resulting truncated protein preserves some enzyme activity and is able to function at 33% of the normal level. Optico-pharyngeal muscular atrophy is being studied in a large family originating from Bukhara, Central Asia. This disease in another large family has previously been linked to a locus on chromosome 14q; our data excluded linkage of the Bukhara family to this locus suggesting genetic heterogeneity. In hereditary inclusion-body myopathy, the deposits in the vacuolated muscle fibers were immunocytochemically identified as Aβ amyloid of Alzheimer's type or admixture of Aβ and prion protein (PrP) amyloids. Complete sequencing of the β-amyloid portion of the APP gene in four patients and PRNP coding region in two affected individuals did not reveal any abnormalities. A genome-wide search is being performed with three large American families with essential tremor, with the use of highly polymorphic minisatellite markers.

*Formerly the Neurogenetics Section.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02875-05 MNB*
PERIOD COVERED October 1, 1994, through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Studies of the Spongiform Encephalopathies and Other Dementing Illnesses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	Lev G. Goldfarb, M.D., Ph.D.	Chief, Clinical Neurogenetics Unit, MNB, NINDS
Other	Olavo Vasconcelos, M.D.	Visiting Fellow CNU, MNB, NINDS
	Tinatin Chabrashvili, M.D.	Visiting Fellow CNU, MNB, NINDS
	Mark Dubnick, Ph.D.	Senior Staff Fellow CNU, CNP, NINDS
	James Nagle, B.S.	Biologist CNU, CNP, NINDS
	Joab Chapman, M.D., Ph.D.	Special Volunteer CNU, MNB, NINDS
	Shira Chapman, M.Sc.	Special Volunteer CNU, CNP, NINDS
COOPERATING UNITS (if any) Paul Brown, M.D., Larisa Cervenakova, M.D., D. Carleton Gajdusek, M.D., LCNSS, Amos Korczyn, M.D., Tel Aviv Univ; V.P. Alexeev, M.D., F.A. Platonov, M.D., S. Kononova, Ph.D., VE Center, Yakutsk		
LAB/BRANCH Medical Neurology Branch, Clinical Neuroscience Program, DIR, NINDS		
SECTION Clinical Neurogenetics Unit		
INSTITUTE AND LOCATION National Institute of Neurological Disorders and Stroke, Bethesda, Maryland		
TOTAL STAFF YEARS:	2.25	PROFESSIONAL: 1.75 OTHER: 0.5
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Mutations and polymorphisms at 25 different sites of the PRNP coding region have been identified (10 of them by this group). In 1995, two new mutations causing familial Creutzfeldt-Jakob disease (CJD) (R3-4 deletion and a point substitution at codon 219) and two new alleles associated with fatal familial insomnia (FFI) (178N + 129M + delR3/4 and 200K + 129M) were discovered and corresponding phenotypes characterized. In FFI and familial CJD, clinically and pathologically distinct syndromes both linked to the 178N mutation, phenotypic expression is dependent on a polymorphism at codon 129: the 178N + 129M allele is responsible for FFI, whereas 178N + 129V causes CJD. The fact that an unique allele, 178N + 129M + delR3/4, produced typical features of FFI confirms that the 178N + 129M alleles are specific for FFI. Homozygosity for either amino acid at position 129 has been identified as a predisposing factor in iatrogenic CJD. Homozygosity for valine at 129th residue may control susceptibility to kuru. In familial CJD, homozygosity for the 129th amino acid has an aggravating effect. The PrP gene in five species of primates used in studies of experimental transmission, shows 95 to 99% sequence homology with the coding region of the human PRNP gene which may explain the varying success in transmitting human disease. DNA polymorphisms at two positions in rhesus monkeys may play an important role in determining sensitivity to an experimental infection. A polymorphism in the apolipoprotein E gene controlling the age of onset in sporadic Alzheimer's disease does not influence the age of onset or the rate of progression in CJD; conversely, codon 129 polymorphism in the PRNP gene does not influence phenotypic expression of other neurological disorders. The gene location for very-early-onset Alzheimer's disease with rapid progression and myoclonus in a Finnish family has been assigned by linkage analysis to chromosome 14q. Apolipoprotein H (Apo H) was shown to be involved in CNS autoimmune disease and its expression increases with aging; we have identified several unknown species of ApoH cDNA in human brain libraries and are currently selecting clones for sequencing analysis. Studies of Viliuisk encephalomyelitis (VE) have been significantly intensified. A variety of laboratory animals have been inoculated with materials from patients with acute and subacute VE in order to isolate and study the infectious agent. </p>		
*Formerly Neurogenetics Section.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02792-07 MNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological Investigations of Human Cognition and Mood State

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Grafman, Ph.D.

Chief

CNS, MNB, NINDS

Others:

M. Stark, Ph.D., IRTA Fellow, CNS, MNB, NINDS

K. Clark, Ph.D., Psychol., MNB, NINDS

T. Rickard, Ph.D., IRTA Fellow, CNS, MNB, NINDS

K. Wild, Ph.D., Psychol., MNB, NINDS

D. Kimberg, IRTA Fellow, CNS, MNB, NINDS

B. Fantie, Ph.D., A.U., Dept. of Psych.

S. Flitman, M.D., Clin. Assoc., CNS, MNB, NINDS

J. Wachs, Ph.D., Psychol., MNB, NINDS

A. Partiot

Spec. Vol., CNS, MNB, NINDS

C. Hollnagel, Spec. Vol., MNB, NINDS

M. West, Spec. Vol., MNB, NINDS*

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Cognitive Neuroscience Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

15.4

PROFESSIONAL:

15.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Current studies in the Cognitive Neuroscience Section focus on planning, thinking, and reasoning; memory and amnesia; visual attention, spatial perception, and object recognition; and emotion/social cognition. Both single-case and group design studies are used. Normal controls, inpatients, and outpatients with central nervous system impairment are recruited for studies. Planning, thinking and reasoning are studied in experiments focusing on schema development, the generation of cognitive plans, analogical thinking, script event generation and verification, number processing and calculation, knowledge representation, and divided resources. Memory and amnesia is studied in experiments focusing on implicit and explicit encoding and retrieval; priming; autobiographic recall; discourse processing, naming and word retrieval, and categorization tasks. Visual attention, spatial perception, and object recognition are studied in experiments focusing on spatial frequency, contrast sensitivity, object knowledge and feature verification, visual-spatial localization, spatial, selective, and sustained attention, and local-global properties of stimuli. Emotion and social cognition are studied in conjunction with cognitive experiments examining attention and memory, rule retrieval, and inhibition. The development of theoretically valid and testable models of cognitive processing is a primary aim of the Section. We study patients with focal and degenerative lesions in order to topographically map components of cognitive processing to brain regions and systems. Pharmacologic challenge and infusion studies are done to evaluate the dissociability of hypothesized components of cognitive processing. Transcranial magnetic stimulation, functional magnetic resonance imaging, positron emission tomography, and event-related brain potentials are all employed to examine the topographic location and computational properties of cognitive components.

*continued:

K. Epstein, A. Rutstein, M. Cantwell, A. Herrmann, K. Knutson, V. Cooper, J. O'Grady, A. Soares, M. Apostolova, M. Petterson, D. Sihweil, D. Williams: Special Volunteers, MNB, NINDS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02711-10 MNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Utility and Physiology of Botulinum Toxin for Involuntary Movement Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.,	Chief	MNB, NINDS
Others:	Barbara I. Karp, M.D.	Chief, Consultation Service	OCD, NINDS
	Ali Samii, M.D.	Visiting Associate	HMCS, MNB, NINDS
	Stephen Grill, M.D., Ph.D.	Senior Clinical Investigator	HMCS, MNB, NINDS

COOPERATING UNITS (if any)

Speech Pathology Unit, NIDCD

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.7

PROFESSIONAL:

0.3

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been studying the efficacy of local injections of botulinum toxin (btx) for the treatment of movement disorders including dystonia, and tremor. When injected in small doses directly into the muscle, botulinum toxin is taken up into the presynaptic neuron at the neuromuscular junction where it prevents the release of acetylcholine. Abnormal muscle contraction is decreased and function improves. Treatment with botulinum toxin is well-tolerated with minimal side effects. We have also been using botulinum toxin to study the physiology of dystonia.

We are completing a trial of btx type F for patients with a loss of response to type A due to antibodies. Eighty-five percent had benefit from btx F which was similar to their previous response to type A. One patient with no benefit from type A also had no benefit from type F. A single patient with loss of response but negative antibody testing for type A toxin, had response to type F injection.

Over 80% of patients with hand dystonia benefit from botulinum toxin injection. The results of a study on the effects of exercise immediately after botulinum toxin injection are being analyzed to see if exercise enhances btx uptake into active neurons with improvement in the extent or duration of benefit.

Eighteen patients with tremor (arm tremor 10, head/neck tremor 8) have been treated to date. Approximately 60% of the tremor patients have more than minimal improvement with btx injection. Data are being collected to assess objectively tremor response to treatment with triaxial accelerometry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02712-10 MNB																		
PERIOD COVERED October 1, 1994 through September 30, 1995																				
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Noninvasive Stimulation of Human Central Nervous System																				
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.: Mark Hallett, M.D.</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">MNB, NINDS</td> </tr> <tr> <td colspan="3">Others:</td> </tr> <tr> <td>Eric Wassermann, M.D.</td> <td>Clinical Investigator</td> <td>OCD, NINDS</td> </tr> <tr> <td>Ali Samii, M.D.</td> <td>Visiting Associate</td> <td>HMCS, MNB, NINDS</td> </tr> <tr> <td>Katsunori Ikoma, M.D.</td> <td>Special Volunteer</td> <td>HMCS, MNB, NINDS</td> </tr> <tr> <td>Bruno Mercuri, M.D.</td> <td>Visiting Fellow</td> <td>HMCS, MNB, NINDS</td> </tr> </table>			P.I.: Mark Hallett, M.D.	Chief	MNB, NINDS	Others:			Eric Wassermann, M.D.	Clinical Investigator	OCD, NINDS	Ali Samii, M.D.	Visiting Associate	HMCS, MNB, NINDS	Katsunori Ikoma, M.D.	Special Volunteer	HMCS, MNB, NINDS	Bruno Mercuri, M.D.	Visiting Fellow	HMCS, MNB, NINDS
P.I.: Mark Hallett, M.D.	Chief	MNB, NINDS																		
Others:																				
Eric Wassermann, M.D.	Clinical Investigator	OCD, NINDS																		
Ali Samii, M.D.	Visiting Associate	HMCS, MNB, NINDS																		
Katsunori Ikoma, M.D.	Special Volunteer	HMCS, MNB, NINDS																		
Bruno Mercuri, M.D.	Visiting Fellow	HMCS, MNB, NINDS																		
COOPERATING UNITS <small>(if any)</small> Speech and Voice Pathology Unit, NIDCD																				
LAB/BRANCH Medical Neurology Branch, CNP, DIR																				
SECTION Human Motor Control Section																				
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892																				
TOTAL STAFF YEARS <div style="text-align: right;">5.4</div>	PROFESSIONAL: <div style="text-align: right;">4.75</div>	OTHER: <div style="text-align: right;">0.65</div>																		
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td colspan="2"></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td colspan="2"></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews											
<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither																		
<input type="checkbox"/> (a1) Minors																				
<input type="checkbox"/> (a2) Interviews																				
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>We continued improving techniques for <u>topographic mapping</u> of <u>motor cortex</u> using <u>transcranial magnetic stimulation (TMS)</u>. We found that motor maps enlarge with acquisition of skills and <u>motor learning</u>, unveiling the role of the primary motor cortex in these cognitive processes. Training to change the timing of execution of two motor acts can enhance their cortical motor representations leading to greater overlapping and suggesting timing is a powerful trigger of <u>cortical plasticity</u>. Patients with cortical <u>strokes</u> (who have recovered) have larger maps of motor output targeting the affected side of the body and lower thresholds for excitation.</p> <p>Cortical motor excitability is increased in <u>dystonia</u> and maps of motor outputs are deranged in the hemisphere contralateral to the dystonic side, a mechanism that could explain the overflow of motor activity in these patients. Magnetic but not electrical stimulation reset postural tremor in <u>Parkinson's disease</u> and <u>essential tremor</u> suggesting an important role of intracortical structures in their generation. Post-exercise facilitation (PEF) of MEPs is decreased while postexercise depression (PED) is not affected in patients with <u>chronic fatigue syndrome</u> and <u>depression</u>.</p> <p>Repetitive transcranial magnetic stimulation (rTMS) delivered over the <u>supplementary motor area (SMA)</u> induces disruption of complex motor sequences with a longer latency than rTMS delivered over primary motor cortex, suggesting an important role for midline structures in advanced planning and performance of complex motor acts. Our studies also suggest a role of the SMA in modulation of segmental reflexes (<u>H-reflexes</u>). We found that the hand may have two ipsilateral cortical representations, one of which, upon stimulation, produces silent periods and the other motor evoked potentials at the same stimulus intensity.</p> <p><u>Verbal recall</u> was consistently diminished after left midtemporal and bilateral dorsofrontal rTMS. rTMS delivered to the right lateral prefrontal area produces <u>mood</u> elevation and increases in TRH secretion in normal subjects and clinical improvement in patients with refractory depression.</p>																				

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02667-11 MNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Analysis of Involuntary Movements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Chief	MNB, NINDS
Others:	Camilo Toro, M.D.	Visiting Scientist	HMCS, MNB, NINDS
	Barbara Karp, M.D.	Chief, Consultation Service	OCD, NINDS
	Stephen Grill, M.D., Ph.D.	Clinical Associate	HMCS, MNB, NINDS
	Ali Samii, M.D.	Visiting Associate	HMCS, MNB, NINDS
	Mary K. Floeter, M.D.	Senior Staff Fellow	OCD, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	2.25	PROFESSIONAL:	1.05	OTHER:	1.2
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Involuntary movements have been classified primarily on descriptive basis. The use of physiological criteria for the classification and study of patients with movement disorders has lead to new insights onto pathophysiology and treatment of these disorders.

We continue our efforts to characterize the physiological mechanisms responsible for positive and negative myoclonus. We have expanded the physiological features of the opsoclonus-myoclonus syndrome. The myoclonus in patients with Gaucher's disease has been studied physiologically.

We have studied physiologically patients with epilepsia partialis continua (EPC) and these findings have been correlated with PET studies these same patients.

Using EEG dsynchronization to voluntary movements and sensory evoked potentials, we have identified differences in the patterns of cortical activation in patients with hand dystonia. These findings support findings from parallel PET and transcranial magnetic stimulation studies. In some patients with tic disorders, we have found patterns of cortical activation similar to those accompanying normal voluntary movements.

We have found abnormalities of spinal cord mechanisms for vibratory inhibition of the H-reflex in patients with stiff-man syndrome (SMS) which implicates a dysfunction of GABAergic spinal cord mechanisms. Patients with hereditary hyperekplexia, a genetic disorder affecting glycine receptors, show abnormalities in reflex pathways thought to be mediated by glycinergic Ia interneurons.

Oligosynaptic spinal cord reflexes including vibratory inhibition, flexor reflexes and cutaneous silent period in patients with severe dystonia before and after intrathecal baclofen are being studied.

The physiological properties of tremor to various inertial loads and during writing were studied in patients with writing tremor before treatment with botulinum toxin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02669-11 MNB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Physiological Analysis of Voluntary Movement		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: Mark Hallett, M.D. Chief MNB, NINDS Others: C. Toro, M.D. Vis. Sci., HMCS, MNB B. Mercuri, M.D., Vis. Fell., HMCS, MNB A. Samii, M.D. Vis. Assoc., HMCS, MNB S. Grill, M.D., Ph.D., Sr. Clin. Assoc., HMCS, MNB M. Deiber, M.D., Ph.D.; G. Resear, HMCS, MNB S. Massaquoi, M.D., Sr. Clin. Assoc., HMCS, MNB V. Ibanez, M.D. Vis. Assoc., HMCS, MNB E. Wassermann, M.D., Clin. Invest., OCD, NINDS N. Sadato, M.D. Vis. Fell., HMCS, MNB		
COOPERATING UNITS (if any) Department of Rehabilitation Medicine, Clinical Center Department of Nuclear Medicine, Clinical Center		
LAB/BRANCH Medical Neurology Branch, CNP, DIR		
SECTION Human Motor Control Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 12.15	PROFESSIONAL: 10.05	OTHER: 2.1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>To understand processes underlying the performance of <u>complex movements</u>, we are studying sequential movements of differing length. In a study with <u>PET</u> we determined that <u>premotor cortex</u> was most related to increasing length of the sequence. With <u>EEG</u>, greater increases in <u>coherence</u> between central and frontal regions were detected for more complex finger movements. To understand <u>motor learning</u>, we have studied the role of sensorimotor areas in the acquisition of implicit and explicit knowledge in a sequential reaction time task using EEG. There was maximal desynchronization of the alpha activity over the contralateral sensorimotor area at the time of development of full explicit knowledge. Using <u>functional MRI</u> (fMRI), we studied the participation of <u>supplementary motor area</u> (SMA), and <u>anterior cingulate cortex</u> (ACC) in self-paced versus externally-triggered motor actions during sequential movements. Performance of simple sequential distal movements activates the SMA and ACC, with a larger change of signal intensity in self-paced than in triggered movements.</p> <p>In <u>blind</u> people, during <u>Braille</u> reading, activation has been found in the primary occipital area using both PET and fMRI. This indicates remarkable <u>cortical plasticity</u>.</p> <p>Patients with <u>Parkinson's disease</u> scale their movements so that they always produce a movement smaller than desired. Studies demonstrated that this might be due to an impairment in <u>kinesthesia</u>.</p> <p>Research efforts have focussed on the role of sensory feedback in the control of movements in patients with <u>cerebellar degenerations</u>. A multiinput-multioutput feedback control system is capable of modelling the response of the standing human body to impulsive perturbations applied to the back. The ability of patients with cerebellar degeneration to perceive differences in kinesthetic stimuli was found to be defective in tasks that were entirely passive. Studies using a task requiring <u>multijoint coordination</u> have been completed which suggest that deficits in motor performance in cerebellar patients is largely due to prolonged <u>reaction times</u>. A <u>clinical rating scale</u> for ataxia has been developed which correlates with the degree of cerebellar abnormality on anatomic and <u>magnetic resonance spectroscopic imaging</u>.</p> <p>We studied patients with <u>hereditary hyperekplexia</u> to see if reflexes thought to be mediated by <u>glycine</u> were affected. Reflex pathways thought to be mediated by glycinergic Ia interneurons were abnormal, but not <u>Renshaw cells</u>, suggesting possible compensatory changes. Two reflex pathways thought to be mediated by <u>GABA</u> were normal.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02630-12 MNB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical, Genetic and Biochemical Studies of Familial Alzheimer's Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Interim P.I.: L.E. Nee, M.S.W. Social Science Analyst CNU, MNB, NINDS		
COOPERATING UNITS (if any) Lev Goldfarb, M.D., NINDS; Jordon Grafman, Ph.D., NINDS; Jay Robbins, M.D., NCI		
LAB/BRANCH Medical Neurology Branch		
SECTION Clinical Neurogenetics Unit		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The <u>gene</u> for <u>familial Alzheimer's disease</u> has been identified for the three major families I have been following. I have continued longitudinal data collection, expansion of pedigree information and recruitment of additional family members, as well as counseling of numerous families some followed since 1977. Collaborations have also continued. In September 1993, we started admitting family members with the collaboration of Trey Sunderland, M.D., NIMH.</p> <p>Emphasis will now be to compare longitudinal study findings, for example LP's PET, infusions, and psychological testing, with DNA findings. A presymptomatic testing projection is being considered.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02531-14 MNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies in Neuromuscular and CNS Diseases and Their Experimental Models

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.C. Dalakas, M.D.	Chief, NDS	MNB, DIR, NINDS
OTHERS:	M. Monzon, Ph.D.	Special Volunteer, NDS	MNB, DIR, NINDS
	I. Illa, M.D., Ph.D.	Exchange Scientist, NDS	MNB, DIR, NINDS
	E. Cupler, M.D.	Clinical Associate, NDS	MNB, DIR, NINDS
	C. Semino-Mora, M.D.	Special Volunteer, NDS	MNB, DIR, NINDS
	N.D. Epstein, M.D.	Molecular Biologist	CHB, DIR, NHLBI

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Neuromuscular Diseases

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.9

PROFESSIONAL:

3.65

OTHER:

2.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Section runs the Laboratory of Muscle Enzyme Histochemistry that processes up to 200 muscle and nerve biopsies per year for diagnostic and research studies. Examined muscles are from patients with: neuromuscular manifestations related to systemic, autoimmune, viral, metabolic, endocrine or infectious diseases; primary neuromuscular disorders, such as polymyositis, dermatomyositis, neurogenic muscular atrophies, muscular dystrophies, post-polio syndrome, polyneuropathies, mitochondrial encephalomyopathies; and patients with biochemical and genetic muscle diseases, such as central core disease or hypertrophic cardiomyopathy. The laboratory is also involved in the following immunological, biochemical and virological studies that examine the susceptibility of the muscle and nerve to immune or viral mediated injuries: (a) study the regeneration of human muscle in health and disease and the maturation of satellite cells by examining the expression of neural cell adhesion molecules and laminins; (b) study the susceptibility of muscle and nerve to infection with retroviruses and the ability of HIV and HTLV-I or HIV and HTLV-I-infected lymphoid cells to infect human myotubes in culture and induce expression of new surface antigens; c) study the expression of the poliovirus receptor in human muscle *in vivo* and *in vitro*, the ability of the poliovirus to infect and replicate in human myotubes and the mechanism of apoptotic cell death induced by the poliovirus; (d) study the effect of cytokines and lymphokines on human myotubes and examine *in vitro* if potentially therapeutic agents such as IVIg can inhibit their toxic or immunopotentiating effect; (e) examine the role of ICAM-I in enhancing myocytotoxicity *in vivo* and *in vitro* by promoting the adhesion of cytotoxic T cells to myotubes; (f) study the toxicity of AZT to muscle mitochondria, mitochondrial oxidative phosphorylation and mitochondrial DNA by applying AZT to human muscle in culture; (g) study the effect of L-carnitine in reversing the mitochondrial abnormalities induced by AZT on human myotubes *in vitro*; and (h) use animal models to study: (i) the pathogenesis of retrovirus-induced inflammatory myopathy by examining muscles from monkeys infected with the simian immunodeficiency virus; (ii) the mechanism of AZT-induced mitochondrial myopathy by examining the structural, metabolic and functional alterations in the muscle mitochondria of healthy rats injected with AZT; (iii) the effect of L-carnitine in reversing or improving the AZT-induced myopathy in the rats; and (iv) the mechanism by which dideoxycytidine induces neurotoxicity in healthy rats.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02038-23 MNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Combined Clinical, Viral and Immunological Studies of Neuromuscular Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.C. Dalakas, M.D., Chief, NDS, MNB, DIR, NINDS

Others:

E. Cuppler, M.D., Neurologist, NDS, MNB, DIR, NINDS

M. Agboatwalla, M.D., Child Specialist,
Karachi, Pakistan

M. Monzon, Ph.D., Spec. Vol., NDS, MNB, DIR, NINDS

B. Sonies, Ph.D., Speech Pathol., CC, DIR, NINDS

L. Goldfarb, M.D., Director, DNA Seq. Fac., DIR, NINDS

K. Sivakumar, M.D., Neurologist, NDS, MNB, DIR, NINDS

A. McLaughlin, Ph.D., DRRP, OD

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Neuromuscular Diseases

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

7.25

PROFESSIONAL:

3.85

OTHER:

3.4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical and laboratory studies are conducted to determine etiology (infection, immunity and/or genetics) of chronic diseases of the neuromuscular system and design effective therapies. Current studies involve patients with polymyositis/dermatomyositis, post-polio syndrome, amyotrophic lateral sclerosis (ALS), demyelinating polyneuropathies, neuromuscular diseases associated with HIV infection, hypokalemic periodic paralysis and Duchenne muscular dystrophy. The pathogenesis of post-polio syndrome is explored with a series of electrophysiological, virological, immunological and histological studies. The findings are compared with those seen in patients with acute paralytic poliomyelitis and other motor neuron diseases. Persistent or mutant poliovirus is sought in these patients' tissues using tissue cultures, PCR, and *in situ* hybridization. Because abnormal immunoregulation was found in some patients, a double-blind placebo-controlled trial using prednisone was conducted. The mechanism of post-polio fatigue, a common and disabling symptom in many patients, is examined by analysis of the neuroendocrine axis and by magnetic resonance spectroscopy. Sequence of the β amyloid precursor protein gene is performed in patients with familial and sporadic inclusion body myositis. The spectrum of neuromuscular disorders associated with HIV infection has been studied and the role of the virus in the cause of neuropathy or myopathy is investigated with a variety of immunocytochemical studies, *in situ* hybridization and PCR. The antiretroviral drug AZT was found to cause a unique myopathy characterized by abnormal mitochondria as determined by various morphological, molecular, biochemical and immunocytochemical studies. A longitudinal study of HIV-positive patients that develop myopathic symptoms while on AZT is conducted with serial muscle biopsies to assess factors associated with the development of myopathy. Patients with AZT-myopathy were found to have low muscle carnitine level. This has prompted the conduction of an ongoing randomized controlled clinical trial using oral L-carnitine. Randomized-controlled clinical trials are conducted with high-dose Intravenous immunoglobulin in patients with polymyositis/dermatomyositis, chronic inflammatory and paraproteinemic demyelinating polyneuropathies, ALS, and Duchenne muscular dystrophy. A controlled study using dichlorophenamide, a carbonic anhydrase inhibitor, is also conducted in patients with hypokalemic periodic paralysis.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02891-03 NEB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Multiple Births and Cerebral Palsy, the International Cooperative Study*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D. Acting Chief NEB, DIR, NINDS

COOPERATING UNITS (if any)

Beverly Petterson, Ph.D., University of Western Australia; Jonas Ellenberg, Ph.D., WESTAT

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.9

PROFESSIONAL:

0.7

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in defined populations in California and Western Australia, and the NCPP, have observed an excess of neurologic morbidity in children born of multiple gestations. Since multiple gestations are increasing in developed countries due to assistive reproductive interventions, and cerebral palsy in twins and triplets now contributes a larger proportion of total cerebral palsy than in the past, we have undertaken a large international study of this problem. Dr. Beverly Petterson of the University of Western Australia was in the NEB for six months during this fiscal year, and has assembled ten datasets, from Sweden, Scotland, from Oxford, Bristol, London, and the Mersey region of Britain, and Victoria, and South Australia, in addition to the California and Western Australian data, a total of 10 datasets and about 2.5 million livebirths among whom we anticipate there will be about 400 children born of multiple gestations who have cerebral palsy. Work to date has established that indeed multiple births have increased during the decade of the 1980s, and that unlike-sex pairs have increased among total twins, consistent with the view that it is pharmacologic and other medical interventions for conception (which techniques produce chiefly dizygotic twins) that have been associated with the increase in twinning and in higher order multiple births. Assembling and preparing this large multiple dataset for analysis is still underway. Dr. Petterson presented preliminary data at an international meeting on twins in Richmond, VA, in June, 1995. Dr. Jonas Ellenberg, who has left the NICHD, will continue to participate in preparation and analyses of these data. Upon her return to Western Australia, Dr. Petterson will work to finish the assembling of the data and will return to the NEB for a month early in 1996 to complete this project and prepare manuscripts describing the results.

*Previously titled "Multiple Births and Cerebral Palsy in Western Australia".

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02892-03 NEB

PERIOD COVERED

October 1, 1994 through September 30 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The EEG as a Predictor in Febrile Seizures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D.

Acting Chief

NEB, DIR, NINDS

Others:

Jonas H. Ellenberg, Ph.D.

Chief

BFSB, DIR, NINDS

Deborah Hirtz, M.D.

Medical Officer

DNB, NINDS

COOPERATING UNITS (if any)

Nikola Sofijanov, M.D., Milutin Dukovski, M.D., Marija Kuturek, M.D., Pediatric Clinic of the University of Skopje, Macedonia (Former Yugoslavia)

LAB/BRANCH

Neuroepidemiology Branch and Biometry and Field Studies Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.01

PROFESSIONAL:

0.01

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

From a population study in Macedonia, all children with febrile seizures were evaluated in one child neurology unit, histories, physical examinations, and electroencephalograms (EEGs) recorded, and children followed for two years. An initial paper described the experience at the first visit, a subsequent manuscript describing the EEG as a predictor of recurrence has been submitted.

This study has been completed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02863-04 NEB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The California Cerebral Palsy Project

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D.

Acting Chief

NEB, DIR, NINDS

COOPERATING UNITS (if any)

Dr. Judith Grether, Birth Defects Monitoring Program, Department of Health Services, California; Health Officers Association of California

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.4

PROFESSIONAL:

0.8

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has established a population-based registry of children with cerebral palsy (CP) born 1983-1985 in four San Francisco Bay Area counties. Within that population-based study, we are undertaking a number of case-control studies, using controls randomly selected within major birthweight groups, approximately 2 per case. Elements completed in this fiscal year include: (a) study of *in utero* exposure to magnesium sulfate in children born weighing <1500 g (very low birthweight, VLBW), finding that whether administered for maternal preeclampsia or in an effort to halt preterm labor, magnesium was associated with a marked reduction in risk of cerebral palsy (CP). A new case-control study of births 1988-1992 in northern California has been designed to examine this association in greater detail, and a feasibility study for a randomized clinical trial (to be supported by a private foundation) was designed and accepted by the IRB of the proposed center, and is scheduled to begin accepting patients in mid-summer, 1995. The NEB has participated in the design of these two new studies and will participate in analyses and publications. (b) A study of prenatal and perinatal risk factors for CP, finding that primigravidity, close pregnancy spacing, delivery in community level hospitals, and birth soon after mother's admission to hospital were associated with increased risk of CP, preeclampsia (in the US usually treated with magnesium) with markedly reduced risk. Birth in a level one facility within three hours of admission was observed in 24% of VLBW children and in no VLBW control. Chorionitis was associated with CP risk only in children who experienced neonatal seizure, and this combination was observed in 14% of VLBW children with CP (in 25% of those with spastic diplegia) and in no control. Re-analyses with CART and logistic regressions are underway. (c) We have recently completed the first study in a defined population to examine specific fetal heart rate patterns in labor, as recorded on electronic fetal heart rate monitoring, as a possible risk factor for CP, and to consider potential confounders. We observe that multiple late decelerations and decrease in beat-to-beat variability, but neither bradycardia nor tachycardia, was associated with increased risk of CP. That relationship persisted after consideration of five other important risk factors for CP. However, positive predictive value was very low. Manuscript has been completed. (d) Data concerning twins from the California project have been merged with nine other population-based datasets on multiple gestations for a study of neurologic morbidity in multiple births, described elsewhere. A variety of other substudies are underway in this data set.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02866-04 NEB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Reliability of Diagnoses of First Seizures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

P.I.: Joseph M. Scheller, M.D. Special Expert NEB, DIR, NINDS

Others: Karin B. Nelson, M.D. Acting Chief NEB, DIR, NINDS

COOPERATING UNITS (if any)

S. Weinstein, M.D., Neurology Department, Children's Hospital National Medical Center.
J. Chamberlain, M.D. Neurology Department and Emergency Medical Trauma Center, CHNMC

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.01

PROFESSIONAL: 0.01

OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An abstract from this study was presented at the American Academy of Neurology annual meeting in 1994.

This project has been completed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02867-04 NEB

PERIOD COVERED

October 1, 1994 through September 30 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurologic Morbidity and its Antecedents Within the NCPP Dataset

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Jonas Ellenberg, Ph.D.
Karin B. Nelson, M.D.Chief
Acting ChiefBFSB, DIR, NINDS
NEB, DIR, NINDS

COOPERATING UNITS (if any)

BFSB, NINDS; NEB, NINDS

LAB/BRANCH

Biometry and Field Studies Branch and Neuroepidemiology Branch,

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.01

PROFESSIONAL:

0.01

OTHER:

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Twinning is increasing in the United States and elsewhere, and recent experience in the California Cerebral Palsy Project observed, and a subsequent study in Western Australia confirmed (both of these with an NEB scientist as participant) that cerebral palsy is more common in twins than in singletons and that cerebral palsy in twins now constitutes a larger proportion of all cerebral palsy than was true in the past. We undertook a study of twins in the NCPP because that dataset has certain advantages: good ascertainment of obstetric complications, recorded from the time of the first prenatal visit in twins and singletons, both those who turned out to have cerebral palsy or seizures and those who did not; information on zygosity on affected and unaffected twins; and information on several outcomes including in utero and neonatal death, and cerebral palsy, (CP) seizure disorders, and tested intelligence. In a total of 52,364 livebirths, there were 1079 twins. Although certain complications of pregnancy were more common in twins, they were not more common in twins with CP. Neonatal and febrile seizures occurred with similar frequency in twins as in singletons. Twins were much more likely to be low in birthweight, but twins <2500 g were not at higher risk for CP than singletons of that birthweight group, while in large twins the risk of CP exceeded that in large singletons. The risk of CP and of nonfebrile seizure disorders was similar in monozygous and dizygous pairs, implying that there must be pathogenetic mechanisms other than the disorders of placentation known to complicate monozygous twinning. Death of one twin was associated with higher rates of CP and of nonfebrile seizures in children without CP, but not with lower intelligence in those without CP or seizures. Low birthweight and *in utero* death of co-twin appear to be the dominant predictors of childhood neurologic disability in twins. A paper on this subject was published in this year.

This study is now completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02838-05 NEB												
PERIOD COVERED October 1, 1994 through September 30, 1995														
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Retroviral Diseases of the Nervous System														
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">co-P.I.:</td> <td style="width: 33%;">Karin B. Nelson, M.D.</td> <td style="width: 33%;">Acting Chief</td> <td style="width: 33%;">NEB, DIR, NINDS.</td> </tr> <tr> <td></td> <td>Clarence J. Gibbs Jr, Ph.D.</td> <td>Deputy Chief</td> <td>CNNS, DIR, NINDS</td> </tr> <tr> <td>Others:</td> <td>Aurora K. Pajeau, M.D.</td> <td>Clinical Associate</td> <td>NEB, DIR, NINDS</td> </tr> </table>			co-P.I.:	Karin B. Nelson, M.D.	Acting Chief	NEB, DIR, NINDS.		Clarence J. Gibbs Jr, Ph.D.	Deputy Chief	CNNS, DIR, NINDS	Others:	Aurora K. Pajeau, M.D.	Clinical Associate	NEB, DIR, NINDS
co-P.I.:	Karin B. Nelson, M.D.	Acting Chief	NEB, DIR, NINDS.											
	Clarence J. Gibbs Jr, Ph.D.	Deputy Chief	CNNS, DIR, NINDS											
Others:	Aurora K. Pajeau, M.D.	Clinical Associate	NEB, DIR, NINDS											
COOPERATING UNITS <small>(if any)</small> Clarence J. Gibbs Jr, Ph.D., DIR, CNSS, NINDS; William A. Blattner, M.D., C. DCE, EEB, NCI.														
LAB/BRANCH Neuroepidemiology Branch														
SECTION														
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892														
TOTAL STAFF YEARS: 0.11	PROFESSIONAL: 0.01	OTHER: 0.10												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> <input checked="checked" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </td> <td style="width: 33%; vertical-align: top;"> <input type="checkbox"/> (b) Human tissues </td> <td style="width: 33%; vertical-align: top;"> <input type="checkbox"/> (c) Neither </td> </tr> </table>			<input checked="checked" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither									
<input checked="checked" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither												
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>A door-to-door field survey of the prevalence of clinically diagnosed <u>tropical spastic paraparesis</u> (TSP) in St. Catherine Parish, Jamaica, was carried out by Dr. Pajeau in collaboration with the Statistical Institute of Jamaica and the University of the West Indies, Kingston. Serum samples were analyzed at the University of the West Indies for HTLV-I, B-12, folate and treponemal antibodies. Subjects were referred to the University of the West Indies TSP Clinic for follow-up in Phase III of the study. Data are being analyzed for clinical diagnosis, seropositivity, and for other factors in the differential diagnosis. A nested case control study is planned to determine risk factors for development of TSP.</p> <p>This project has been completed.</p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02746-09 NEB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

,Phenobarbital Clinical Trial in Children with Febrile Seizures*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D. Acting Chief NEB, DIR, NINDS

Others: Deborah Hirtz, M.D. Pediatric Neurologist DNB, DCDND, NINDS
Jonas H. Ellenberg, Ph.D. Chief BFSB, DIR, NINDS

COOPERATING UNITS (if any)

Jacqueline Farewell, M.D., Dept. of Neurosurgery, Univ. of Washington, Seattle, WA

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.02 PROFESSIONAL: 0.02 OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of the study are to assess the effects of phenobarbital, a commonly prescribed anticonvulsant, on tests of intelligence and behavior in children. The design of this study permitted comparison of measures of tested intelligence and of behavior in children with febrile seizures who had been treated with phenobarbital, and in a group of seizure-free control children. A comparison of the groups allowed assessment of benefit and risk of treatment for a common childhood neurologic problem.

*[This study supports the DNB/ND/NINDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Deborah G. Hirtz, DNB, DCDND, NINDS, and the contractor of the study is the University of Washington. The primary paper from this study was published in the *New England Journal of Medicine*. This study has been completed.]

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02240-19 NEB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiology of Dementia and Other Neurodegenerative Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Irene Litvan, M.D. Senior Staff Fellow NEB, DIR, NINDS

Others: Karin B. Nelson, M.D. Acting Chief NEB, DIR, NINDS
Darsy Calderon, M.P.H. Special Volunteer NEB, DIR, NINDS

COOPERATING UNITS (if any)

Carlos A. Mangone, M.D., Francisco Santojanni General Hospital, Buenos Aires, Argentina, SA

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.2

PROFESSIONAL:

1.0

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Neuroepidemiology Branch and an international group of expert and nonexpert neurologists evaluated the accuracy in the diagnosis of different Parkinsonian and dementia disorders, including the accuracy of published criteria for the clinical diagnosis of progressive supranuclear palsy (PSP), using clinical material confirmed by postmortem examination. The Neuroepidemiology Branch, the Society for Progressive Supranuclear Palsy, Inc. (SPSP); and an international group of expert neurologists, used the previously described data to develop better clinical criteria for the diagnosis of PSP.

Vascular dementia is the second-most common cause of dementia in the elderly. Analytic studies to determine risk factors for vascular dementia and Alzheimer's disease are being planned to be developed in Argentina in a well-defined population. Although diagnostic accuracy is important in epidemiologic studies, most case-control studies on dementia do not have well-defined populations. Neuroradiological anatomic (MRI) and perfusion imaging (Tc99 HMPAO cerebral SPECT) studies useful to differentiate Alzheimer's disease dementia from vascular dementia will be used in our studies.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02307-19 NEB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Educational Resources in Neurological Epidemiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D.

Acting Chief

NEB, DIR, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.14

PROFESSIONAL:

0.04

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Because there is a severe shortage of available manpower in neuroepidemiology, the Neuroepidemiology Branch (NEB) has given particular attention to junior members of the American Academy of Neurology (Neurology residents). The NEB has participated actively in the annual courses of the American Academy of Neurology, in an effort to increase the interest in neuroepidemiology. The following are some of these activities:

- (1) Full-day course final lecture participation, American Academy of Neurology: "Clinical Research Methods, Summary and Applications" Seattle, Washington. Dr. Nelson was selected Secretary of the American Academy of Neurology Section on Neuroepidemiology.
- (2) World Federation of Neurology, Research Group on Neuroepidemiology Annual Meeting, Seattle, Washington.
- (3) Organization of a symposium on Neurotoxicology, presented at the meeting of the International Child Neurology Association, held conjointly with the Child Neurology Society in early October, 1994.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02853-04 NIB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Examination of Natural History and Therapy of Multiple Sclerosis Using MRI		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Henry F. McFarland, M.D.	Chief NIB DIR NINDS
Others:	Michael K. Racke, M.D.	Senior Clinical Investigator NIB DIR NINDS
	Tanya J. Lehky, M.D.	Senior Assistant Surgeon NIB DIR NINDS
	Lael Stone, M.D.	Clinical Associate NIB DIR NINDS
	Peter Calabresi, M.D.	Clinical Associate NIB DIR NINDS
	Mary E. Smith, M.D.	Senior Clinical Investigator NIB DIR NINDS
COOPERATING UNITS (if any) Joseph A. Frank, M.D., Director, LDDR, OD, Paul Albert, Ph.D., BFS, DIR, NINDS; Charles DeCarli, M.D., Epilepsy Research Branch, NINDS		
LAB/BRANCH Neuroimmunology, CNP		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	3.1	PROFESSIONAL: 1.75 OTHER: 1.35
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>The goal of this project is to use <u>magnetic resonance imaging (MRI)</u> to examine the natural history and effectiveness of experimental therapies in <u>multiple sclerosis (MS)</u>. Emphasis has been placed on investigation of the early MS lesion which is characterized by enhancement on T1- weighted MRI images following administration of <u>gadolinium-DTPA (Gd)</u>. Results from initial studies have indicated that MS can be an active disease, even during periods of remission in the early, relapsing-remitting phase of the illness. Disease activity MRI has been correlated with clinical disease. Episodes of worsening tend to occur during periods of increased disease activity as evidenced by increased frequency or area of enhancing lesions. The clinical symptoms and signs generally are due to lesions occurring in the spinal cord or brain stem concurrently with the increased activity in the cerebrum. Examination of the pathological changes occurring in conjunction with Gd enhancement indicate an acute inflammatory process with prominent perivascular cuffs of lymphocytes. These findings support the hypothesis that Gd enhancement represent the initial step in lesion development.</p> <p>The ability to use MRI as an outcome measure in clinical trials has been examined in a baseline, versus treatment trial design studying IFN-β1B. The results of this study indicate that IFN-β1B has a dramatic effect on the occurrence of gadolinium-enhancing lesions; the frequency of gadolinium-enhancing lesions was dramatically reduced in patients on treatment as compared with lesion frequency prior to treatment. Additional information relating to the natural history of MS has been derived from a study of a 68-patient cohort studied for a minimum of three months by serial MRI. Results of this study indicate approximately two-thirds of patients with early-mild, relapsing-remitting MS have active disease on MRI, again indicating the disease process is active, even during this early-remitting phase. In addition to the occurrence of gadolinium-enhancing lesions, assessment of changes in the overall magnitude of the disease on MRI as measured by the volume of T2 lesions, indicates progression in a population of patients with mild, relapsing-remitting MS.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02831-05 NIB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Regulation of Class I Major Histocompatibility Complex Gene Expression in the Central Nervous System		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
PI:	Paul D. Drew, Ph.D.	Senior Staff Fellow NIB DIR NINDS
Others:	Kevin G. Becker, Ph.D.	Senior Staff Fellow NIB DIR NINDS
	William E. Biddison, Ph.D.	Section Chief NIB DIR NINDS
	Maryrose Franko, Ph.D.	IRTA Fellow NIB DIR NINDS
COOPERATING UNITS <small>(if any)</small> Keiko Ozato, Ph.D., Laboratory of Molecular Growth Regulation, NICHD		
LAB/BRANCH Neuroimmunology Branch, CNP		
SECTION Molecular Immunology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	4.13	PROFESSIONAL: 3.28 OTHER: 0.85
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>The goal of this project is to understand the mechanisms by which transcription factors regulate gene expression in cells of the central nervous system (CNS). Two projects which target this objective have been conducted within the past year:</p> <p>The first project involves an investigation of the molecular mechanisms which regulate MHC class I gene expression in virus-infected cells of the CNS. The principle findings are that measles infection of glial cells results in an induction of MHC class I gene expression. However, the expression of these genes are not induced to virus-infected neuronal cells. The differential expression of MHC class I genes in virus-infected neural cells is explained by a differential induction of IFN-β gene expression in the cells which is mediated by the transcription factor NFkB.</p> <p>The second project involves the rapid cloning and characterization of transcription factors expressed in human brain by a technique that we have termed signature sequencing analysis. This resulted in the isolation of 133 unique human zinc finger cDNAs of which <u>118 are novel</u>. These cDNA clones have a high degree of homology to zinc finger genes that have been associated with neuronal development and tumorigenesis. One of these cDNA clones is the human form of the <i>Xenopus</i> Pol III basal transcription factor <u>TFIIIA</u>.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02817-06 NIB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Involvement of Human Retrovirus Associated with Chronic Neurologic Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Steven Jacobson, Ph.D.	Section Chief NIB DIR NINDS
Others:	Henry F. McFarland, M.D.	Chief NIB DIR NINDS
	Tanya Lehky, M.D.	Senior Assistant Surgeon NIB DIR NINDS
	Michael Levin, M.D.	Special Volunteer NIB DIR NINDS
	Takeo Kawanishi, M.D.	Visiting Fellow NIB DIR NINDS
COOPERATING UNITS (if any) W. Blattner, M.D., Chief, VES, NCI; G. Shearer, M.D., Section Chief, EIB; T. Waldmann, M.D. Chief, MET Branch, NCI; Anthony Fauci, M.D., Director, NIAID		
LAB/BRANCH Neuroimmunology, CNP		
SECTION Viral Immunology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	4.9	PROFESSIONAL: 3.5 OTHER: 1.4
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>human T lymphotropic virus type I (HTLV-I)</u> is associated with a chronic-progressive myelopathy known as <u>HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP)</u>, a disease clinically similar to the chronic-progressive form of <u>multiple sclerosis (MS)</u>. An understanding of the <u>pathogenesis</u> of a neurologic disease such as HAM/TSP will aid in defining similar mechanisms in MS, a disease of unknown etiology. Five major areas of research are being targeted: (1) the host immune response in the HAM/TSP disease process and the role of CD8+, <u>CTL</u>, and <u>cytokines</u>; (2) the <u>in situ</u> detection of retroviral sequences in the <u>central nervous system</u> and <u>lymphoid organs</u> of HAM/TSP patients; (3) the construction of HTLV-I <u>transgenes</u> that express HTLV-I gene products in a tissue-specific manner; (4) the <u>molecular characterization</u> of potentially novel human retroviruses isolated from patients with other chronic -progressive neurologic diseases, including MS; and (5) immunotherapeutic strategies for the treatment of HAM/TSP. The major findings of these studies are: (1) the demonstration of CD8+, CTL directly isolated from PBL or CSF of HAM/TSP patients that use a limited set of <u>T- cell receptors</u>; (2) the establishment of an <u>ELISPOT</u> assay to quantify the number of cytokine (γ-IFN, IL-2, IL-4) producing cells in HAM/TSP patients PBL and has shown a high frequency in these patients; (3) HTLV-I tax mRNA signals were detected in spinal cord lesions of HAM/TSP patients and have been colocalized to <u>astrocytes</u>; (4) the technique of <u>in situ</u>-PCR has amplified HTLV-I tax DNA from the CNS of HAM/TSP patients and has also demonstrated a large reservoir of HTLV-I-infected cells in <u>bone marrow</u>; (5) an HTLV-I tax construct has been developed under control of an <u>astrocyte-specific promoter</u> in which HTLV-I tax RNA is produced in cells of astroglial origin. <u>Transgenic</u> mice are being developed; (6) strong seroreactivity to HTLV- related antigens has been demonstrated from a number of patients with chronic-progressive myelopathies, and attempts to molecularly identify this potentially novel retrovirus are being pursued; (7) HTLV-II is continuing to be associated with chronic myelopathies; and (8) a phase I/II <u>trial</u> of humanized anti-IL2 receptor antibody in the treatment of HAM/TSP has begun in which immunologic-affects have been demonstrated. These results continue to define the role of human retroviruses that are associated with chronic -progressive neurologic disease. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02603-12 NIB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Mechanisms of Lymphoid Cell-Cell Interactions		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	William Biddison, Ph.D.	Section Chief NIB DIR NINDS
Others:	Ursula Utz, Ph.D.	Visiting Fellow NIB DIR NINDS
	Tomika Tsuchida, M.D., Ph.D.	Visiting Fellow NIB DIR NINDS
	Henry F. McFarland, M.D.	Chief NIB DIR NINDS
	Kiri Honma, M.D., Ph.D.	Visiting Fellow NIB DIR NINDS
	Suchismita Chattopadhyay, Ph.D.	IRTA Fellow NIB DIR NINDS
COOPERATING UNITS (if any) John E. Coligan, Ph.D., Chief, Biological Resources Branch, DIR, NIAID; Eric O. Long, Ph.D. Laboratory of Immunogenetics, NIAID		
LAB/BRANCH Neuroimmunology Branch, CNP		
SECTION Molecular Immunology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	5.22	PROFESSIONAL: 2.82 OTHER: 2.4
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The general objective of this project is to define the mechanisms by which human lymphoid cells interact with antigen-presenting cells in order to produce and regulate immune responses. Over the past year, there have been four major efforts under way that are targeted on this objective: (1) dissection of the molecular basis for <u>peptide binding to class I HLA molecules</u> and presentation for <u>CD8+ T- cell recognition</u>; (2) identification of <u>autoreactive CD8+ T- cell responses to myelin-derived peptides</u>; (3) identification of forms of <u>viral peptide epitopes</u> which can be generated in the endoplasmic reticulum and presented to CD8+ T cells; and (4) identification of peptide/class I HLA complexes recognized by <u>human natural killer cell clones</u>. The principal findings are as follow: (1) isolation and sequencing of endogenous peptides bound to the HLA class I molecules HLA-B14 and HLA-B44 has permitted identification of specific combinations of peptide anchor residues which can be used to successfully predict immunogenic T- cell epitopes within viral peptide sequences that are presented to CD8+ T cells; (2) peptide sequences derived from human myelin basic protein, proteolipid protein, and myelin-associated glycoprotein were identified which could bind to the HLA-A2 molecule and induce autoreactive CD8+ cytotoxic T- lymphocyte responses in MS patients and normal individuals; (3) a viral peptide molecularly designed as a signal sequence could be cleaved from the carrier protein in the endoplasmic reticulum and bound by HLA-A2 and presented to peptide-specific CD8+ T cells on the cell surface; and (4) we are the first group to unequivocally demonstrate that human natural killer cell clones have cell surface receptors that can specifically recognize class I HLA/peptide complexes in a way which is indistinguishable from CD8+ T cells.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02202-20 NIB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunogenetic Studies in Patients with Multiple Sclerosis and Other CNS Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Henry F. McFarland, M.D.	Acting Chief NIB DIR NINDS
Others:	Mary E. Smith, M.D.	Senior Clinical Investigator NIB DIR NINDS
	Tanya Lecky, M.D.	Senior Assistant Surgeon NIB DIR NINDS
	Lael Stone, M.D.	Clinical Associate NIB DIR NINDS
	Steven Jacobson, Ph.D.	Section Chief NIB DIR NINDS
	Peter Calabresi, M.D.	Clinical Associate NIB DIR NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Neuroimmunology, CNP		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.6	PROFESSIONAL: 1.95	OTHER: 1.65
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The overall goal of this project is to assess <u>genetic</u> and <u>immunological</u> factors that contribute to the pathogenesis of neurological disease and to identify therapeutic approaches based on modification of immunological mechanisms. Particular attention is focused on <u>multiple sclerosis (MS)</u>, since this disease is thought to have an immunological basis. Both genetic and immunological factors are being examined in patients with well-characterized, clinically definite MS. Genetic aspects of the disease are being examined both in sporadic patients, as well as, members of multiplex MS families that have multiple affected members, and in sets of monozygotic or dizygotic twins that are concordant or discordant for MS. <u>T-cell receptor (TCR)</u> usage has been examined in sets of monozygotic twins, either concordant or discordant for MS. The results of these studies have shown a general skewing of TCR usage, and have demonstrated increasing diversity of the CDR-3 region with progression of disease. Parallel studies are now being conducted in monozygotic twins with other immunological diseases such as insulin-dependent diabetes mellitus.</p> <p>Various forms of <u>immunomodulatory therapy</u> are being examined in the treatment of MS. The cytokine, transforming growth factor-β2, which has been found to be effective experimentally in the animal model, experimental allergic encephalomyelitis, is now being tested in Phase I studies in patients with active, chronic-progressive MS. The Phase I trial is designed to examine toxicity as a first step in planning larger studies designed to examine efficacy of this treatment.</p> <p>The clinical course and potential treatment of patients with <u>HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP)</u> is being evaluated. This disease may represent an example of <u>viral-induced immunopathological disease</u>, and consequently, represents an important model disease to study immunological parameters and the effect of immunomodulatory therapies. Preliminary testing of a therapy designed to reduce the number of activated T cells using an antibody to the <u>IL-2 receptor</u> are now under way. The study involves monitoring both immunological and clinical parameters.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02204-20 NIB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Mechanisms in Experimental Autoimmune Diseases of the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Henry F. McFarland, M.D.	Chief	NIB	DIR	NINDS
Others:	Mary E. Smith, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS
	Rhonda Voskuhl, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS
	Peter Calabresi, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS

COOPERATING UNITS (if any)

Cedric S. Raine, Ph.D., Prof., Albert Einstein Univ.: Michael J. Leonardo, M.D., Chief, Development of the Immune System Unit; NIAID, Richard M. McCarron, Ph.D., Special Expert, SB, DIR, NINDS

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Neurological Disease Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.53

PROFESSIONAL:

1.33

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	<input type="checkbox"/>	(b) Human tissues	<input checked="" type="checkbox"/>	(c) Neither
<input type="checkbox"/>	(a1) Minors				
<input type="checkbox"/>	(a2) Interviews				

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current work is focused on the chronic-relapsing model experimental allergic encephalomyelitis (EAE). This disease is produced by transfer lymphocytes sensitized to myelin basic protein (MBP) or proteolipid protein to syngeneic mice. Neurologic dysfunction is characterized pathologically by inflammation and primary demyelination. The immunological mechanisms responsible for the initial episode and for the chronic disease are being investigated. Particular attention has focused on the role of activated T cells and regulatory mechanisms occurring at the level of EAE lesion. Recent studies have demonstrated that the administration of soluble MBP given the following cell transfer, or even after the onset of clinical disease, can ameliorate clinical illness. It is thought that the mechanism for this effect is the induction of apoptosis or programmed cell death. T cells which are activated and encounter antigen in the absence of a second signal i.e., IL-2, may be induced to undergo apoptosis.

The effect of treatments which may alter cytokine profiles such as retinoic acid have been studied and found to reduce the severity of diseases. The biological mechanisms of retinoic acid treatment are being examined. To monitor the evolution of the EAE lesion, techniques have been developed to allow MRI studies of the mouse brain. Initial studies indicate that MRI changes correlate with inflammation and demyelination. This technique will be employed to monitor changes in the evolution of the lesion in parallel with immunological studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02205-20 NIB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Interactions Between the Human Immune System and Antigens in the Nervous System		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
PI:	Henry F. McFarland, M.D.	Chief NIB DIR NINDS
Others:	William E. Biddison, Ph.D.	Section Chief NIB DIR NINDS
	Peter Calabresi, M.D.	Clinical Associate NIB DIR NINDS
	Roland Martin, M.D.	Special Volunteer NIB DIR NINDS
	Marco Vergelli, M.D.	Visiting Associate NIB DIR NINDS
	Clara Pelfrey, Ph.D.	IRTA Fellow LI DIR NIAID
COOPERATING UNITS <small>(if any)</small> John R. Richert, M.D., Assoc. Prof., Georgetown U.; Diane Griffin, M.D., Ph.D., Prof., Dept. Neurology, Johns Hopkins U.; Rhonda R. Voskuhl, M.D., Clinical Associate, Molecular Genetics Sections, NINDS		
LAB/BRANCH Neuroimmunology, CNP		
SECTION Cellular Immunology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	7.72	PROFESSIONAL: 6.07 OTHER: 1.65
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unrounded type. Do not exceed the space provided.)</small> <p>The goal of this project is to examine the manner in which immunologic mechanisms may contribute to diseases of the nervous system. The cellular and humoral immune response to putative antigens in possible <u>immunopathologic disease</u> such as <u>multiple sclerosis (MS)</u> are being studied. Included in these studies have been examinations of the immune response to viruses which can commonly infect the nervous system and which could be related to the induction of immunopathologic disease processes. In addition, the immune response to <u>antigens of myelin</u> such as <u>myelin basic protein (MBP)</u> and <u>proteolipid protein (PLP)</u> which may represent targets of immune-mediated diseases of myelin, has been studied. T-cell response to these antigens is being examined in patients with clinically definite MS and healthy controls in members of MS multiplex families, and in identical and nonidentical twins, either concordant or discordant for MS. Various parameters of the T-cell response to myelin antigens is being examined, including the frequency, peptide specificity, and HLA restriction of the T cells.</p> <p>Studies of the T-cell response to the 18.5 kDa form of MBP have indicated several regions of the molecule which appear to be immunodominant. One of these regions defined by amino acids 80-100 has been studied in detail, both with respect to amino acids important for binding of the peptide to the MHC class II antigen-binding cleft, as well as, amino acids important in interacting with the T-cell receptor. Studies have been conducted which based on the modification of amino acids thought to be important in T-cell recognition have altered the T-cell response to this immunodominant peptide. These studies are currently being expanded with the thought that this may form the basis for an immunologically-specific approach to modifying the T-cell response to this MBP epitope. The <u>cytokine</u> profile of T-cells specific for MBP or other myelin antigens is being examined. These studies involve characterization of the cytokine profile for T-cell lines and clones reactive with myelin proteins. In addition to the 18.5 kDa form of MBP, the T-cell response to other myelin antigens and other forms of MBP is being examined. This includes studies of the C-8 form of MBP which has 6 citrulline substitutions for alanine in the 18.5 kd form. Other myelin antigens, including proteolipid protein and <u>CNPase</u> are being studied.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02315-18 NB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Positron Emission Tomography		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	Giovanni Di Chiro, M.D.	Chief NB, CNP, DIR, NINDS
Others:	R.A. Brooks, Ph.D.	Staff Physicist NB, NINDS
	R. Raman, M.D.	Sr. Staff Fellow NB, NINDS
	I. Bicik, M.D.	Special Volunteer NB, NINDS
	D. Laske, M.D.	Medical Staff Fellow SN, NINDS
	C. Ram, M.D.	Visiting Scientist SN, NINDS
	E.H. Oldfield, M.D.	Chief SN, NINDS*
COOPERATING UNITS (if any) NM, CC; BEIP, NCRR; SNB, NINDS; NIDDK.		
LAB/BRANCH Neuroimaging Branch, CNP, DIR		
SECTIONS Clinical Studies and Experimental PET		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS:	0.7	PROFESSIONAL: 0.7 OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Positron emission tomography (PET) is a nuclear medicine technique which allows us to obtain some anatomic information (e.g., axial, coronal or sagittal images of the brain) as well as dynamic functional data (such as regional cerebral glucose consumption rate). The unique property of PET is that it provides physiologic and pathophysiologic information not available with any other imaging procedure. Using a variety of radiopharmaceuticals (mostly ¹⁸ F-deoxyglucose and ¹⁸ F-L-Dopa) as tracers, we have investigated with PET, brain tumors and movement disorders (Parkinson disease, in particular). During the last year we have completed a study on steroids effects on cerebral glucose utilization in patients with brain tumors and found that they reduce the global rate of cerebral glucose consumption.-		
* Continued:		
	C.V. Kufta, M.D.	Staff Physician SN, NINDS
	I. Simpson, M.D.	Associate Chief D, NIDDK
	D. Accili, M.D.	Visiting Scientist D, NIDDK

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02073-22 NB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Nuclear Magnetic Resonance (Imaging and Spectroscopy)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	Giovanni Di Chiro, M.D.	Chief NB, CNP, DIR, NINDS
Others:	R.A. Brooks, Ph.D.	Staff Physicist NB, NINDS
	A. Barnett, Ph.D.	Research Physicist NB, NINDS
	G. Tedeschi, M.D.	Visiting Scientist NB, NINDS
	C Pierpaoli, M.D	Visiting Associate NB, NINDS
	J. Vymazal, M.D., Ph.D.	Visiting Fellow NB, NINDS
	R. Raman, M.D.	Senior Staff Fellow NB, NINDS*
COOPERATING UNITS (if any) <i>In vivo</i> NMR Research Center; NCRR, Diagnostic Radiology Department; DMNB, NINDS; SB, NINDS; ETB, NINDS BEIP, NCRR; Albert Einstein College of Medicine, NY		
LAB/BRANCH Neuroimaging Branch		
SECTIONS		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS:	8.65	PROFESSIONAL: 7.3
		OTHER: 1.35
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Our <u>NMR</u> imaging research is developing along the following lines: a) proton-MR spectroscopic imaging (¹ H-MRSI) in normal controls as well as in patients with tumors, stroke, lipid storage diseases, Parkinson's disease, Alzheimer disease and other degenerative conditions; b) diffusion-perfusion imaging in cerebral ischemia in animal models as well as in patients with stroke; c) analysis of the signal intensity from critical areas (basal ganglia, substantia nigra) in patients affected by a variety of movement disorders; the relationship of the signal intensity with regional iron accumulation is then investigated.		
*Continued:		
N. Lundbom, M.D.	Visiting Associate	NB, NINDS
S. Bonavita, M.D	Special Volunteer	NB, NINDS
A. Bertolino, M.D.	Special Volunteer	NB, NINDS
B. Choi, M.D.	Contract Employee	NB, NINDS
M. Hallett, M.D.	Director	CNP, DIR, NINDS
R. O. Brady, M.D.	Chief	DMNB, NINDS
N. Barton, M.D,	Section Chief	DMNB, NINDS
J.M. Hallenbeck, M.D.	Chief	SB, NINDS
T.J. De Graba, M.D	Medical Researcher	SB, NINDS
T. Chase, M.D.	Chief	ETB, NINDS
R. Schiffmann, M.D.	Senior Staff Fellow	DMNB, NINDS
L Verhagen, M.D.	Visiting Associate	ETB, NINDS
S. Posse, M.D.	Visiting Fellow	DRD
G. Campbell, Ph.D.	Chief	BFSB, NINDS
J. N. Duyn, Ph.D.	Visiting Scientist	LDRR
C. Moonen, Ph.D.	Manager	NMR, NCRR, BEIP
J. Bulte, M.D.	Visiting Fellow	LDRR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02895-02 OCD
PERIOD COVERED October 01, 1994, through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Evaluation of Neuromuscular Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mark Hallett, M.D.	Clinical Director OCD ODIR DIR NINDS
Others:	Carlos Luciano, M.D.	Act. Chief, EMG Lab EMG OCD ODIR DIR NINDS
	Mary Kay Floeter, M.D.	Senior Staff Fellow EMG OCD ODIR DIR NINDS
	James Russell, M.D.	Visiting Associate EMG OCD ODIR DIR NINDS
	Shema Mathew, M.D.	Special Volunteer EMG OCD ODIR DIR NINDS
	Nguyet Dang	Biomedical Engineer EMG OCD ODIR DIR NINDS
	Marinos Dalakas, M.D.	Chief NDS MNB ODIR DIR NINDS
COOPERATING UNITS (if any) National Institute of Arthritis and Musculoskeletal and Skin Diseases; National Cancer Institute		
LAB/BRANCH Office of the Clinical Director, CNP, DIR		
SECTION Electromyography (EEG) Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	2.0	PROFESSIONAL: 1.4 OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This protocol was developed as an effort to characterize new <u>neuromuscular diseases</u> , to learn more about established diseases, to assess current methodologies and technologies and to refine old methods as well as to develop new ones. Using different electrodiagnostic techniques, we have carried on a number of different studies: a. Power spectrum of <u>surface electromyography (EMG)</u> in <u>myopathy</u> : Surface recording of the muscle, EMG may provide a painless alternative to <u>needle electromyography</u> in neuromuscular diseases. Analysis of surface EMG in patients with biopsy-proven myopathy suggests that ratios of the power at low and high frequencies may increase the sensitivity of the test. b. Autonomic studies in <u>Fabry's disease</u> : A basic battery of autonomic testing that can be done in the EMG laboratory, including <u>sympathetic skin responses</u> , <u>Valsalva ratio</u> and <u>heart rate variability</u> with respiration, has been used to study autonomic function in these patients. Preliminary results have shown some abnormalities, primarily with cardiac parasympathetic function. c. Reproducibility of <u>quantitative sensory studies</u> : We have performed serial quantitative sensory studies in a group of healthy volunteers to define the intertrial and intraindividual variability for the different modalities of testing. This test is being used for the evaluation of <u>peripheral neuropathy</u> .		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01424-29 OCD												
PERIOD COVERED October 1, 1994 through September 30, 1995														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Behavioral Modulation by the Limbic System in Man														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">P. Fedio, Ph.D.</td> <td style="width: 33%;">Unit Chief</td> <td style="width: 33%;">CNU, OCD, NINDS</td> </tr> <tr> <td>Others:</td> <td>C. Kufta, M.D.</td> <td>Medical Officer</td> <td>SNB, NINDS</td> </tr> <tr> <td></td> <td>S. Sato, M.D.</td> <td>Medical Officer</td> <td>EEG, OCD, NINDS</td> </tr> </table>			PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS	Others:	C. Kufta, M.D.	Medical Officer	SNB, NINDS		S. Sato, M.D.	Medical Officer	EEG, OCD, NINDS
PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS											
Others:	C. Kufta, M.D.	Medical Officer	SNB, NINDS											
	S. Sato, M.D.	Medical Officer	EEG, OCD, NINDS											
COOPERATING UNITS (if any) Surgical Neurology Branch, DIR, NINDS; Office of the Clinical Director, NINDS														
LAB/BRANCH Office of the Clinical Director														
SECTION Clinical Neuropsychology Unit														
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892														
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The personality profiles of patients with <u>temporal lobe epilepsy</u> (TLE) with <u>mesial</u> or <u>lateral</u> lesions were studied preoperatively with the Millon Clinical Multiaxial Inventory (MCMI). The pattern of traits was not pathognomic, but patients with left and right mesial lesions differed and appeared to be more maladaptive than those with lateral lesions.</p> <p>Patients with left mesial lesions displayed a <u>schizoid</u>, <u>avoidant</u>, <u>anxious</u> and <u>dysthymic</u> profile. Intensity of these traits was influenced by the severity of their language impairment and early onset of seizures. Right mesial patients were more likely immature and <u>histrionic</u>, and intent on preserving a positive image.</p> <p>Long-standing injury to mesial temporal structures, notably the <u>amygdala</u>, alters how the brain codifies and regulates <u>emotional experiences</u> and shapes personal and social self-concept.</p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01245-30 OCD																	
PERIOD COVERED October 1, 1994 through September 30, 1995																			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) EEG Learning Correlates Using Scalp and Intracranial Electrodes																			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"><tr><td style="width: 15%;">PI:</td><td style="width: 30%;">P. Fedio, Ph.D.</td><td style="width: 20%;">Unit Chief</td><td style="width: 35%;">CNU, OCD NINDS</td></tr><tr><td rowspan="4">Others:</td><td>S. Sato, M.D.</td><td>Medical Officer</td><td>EEG, OCD, NINDS</td></tr><tr><td>C. Kufta, M.D.</td><td>Medical Officer</td><td>SNB, NINDS</td></tr><tr><td>M. Schultheis</td><td>Psychologist</td><td>Special Volunteer</td></tr><tr><td>R. Davidson</td><td>Psychologist</td><td>Special Volunteer</td></tr></table>			PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD NINDS	Others:	S. Sato, M.D.	Medical Officer	EEG, OCD, NINDS	C. Kufta, M.D.	Medical Officer	SNB, NINDS	M. Schultheis	Psychologist	Special Volunteer	R. Davidson	Psychologist	Special Volunteer
PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD NINDS																
Others:	S. Sato, M.D.	Medical Officer	EEG, OCD, NINDS																
	C. Kufta, M.D.	Medical Officer	SNB, NINDS																
	M. Schultheis	Psychologist	Special Volunteer																
	R. Davidson	Psychologist	Special Volunteer																
COOPERATING UNITS (if any) Surgical Neurology Branch, DIR																			
LAB/BRANCH Office of the Clinical Director																			
SECTION Clinical Neuropsychology Unit																			
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892																			
TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3																	
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"><tr><td style="width: 33%;"><input checked="" type="checkbox"/> (a) Human subjects</td><td style="width: 33%;"><input type="checkbox"/> (b) Human tissues</td><td style="width: 33%;"><input type="checkbox"/> (c) Neither</td></tr><tr><td><input type="checkbox"/> (a1) Minors</td><td></td><td></td></tr><tr><td><input type="checkbox"/> (a2) Interviews</td><td></td><td></td></tr></table>			<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews										
<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither																	
<input type="checkbox"/> (a1) Minors																			
<input type="checkbox"/> (a2) Interviews																			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Low dosages of amytal injected in patients with <u>temporal lobe epilepsy</u> during the left or right <u>intracarotid amytal (Wada)</u> procedure produced <u>euphoria</u> or <u>dysphoria</u>, respectively. Whether these emotional changes reflected ipsilateral or contralateral release was studied by <u>EEG</u> recordings.</p> <p>Power spectral analysis revealed that patients with left temporal epilepsy had more EEG slowing overall, more so from frontal leads ipsilateral to injection. This preliminary data suggests a mechanism of ipsilateral release of emotions, and that the right brain modulates <u>positive</u>, and the left brain, <u>negative</u> feelings...</p>																			

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 00200-41 OCD

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cognitive and Emotional Profile of Neuropsychiatric Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS
Others:	C. Kufta, M.D.	Medical Officer	SNB, NINDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, DIR

LAB/BRANCH

Office of the Clinical Director

SECTION

Clinical Neuropsychology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	0.5	PROFESSIONAL:	0.4	OTHER:	0.1
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiments using brain stimulation and behavioral procedures were used to identify the neuroanatomic basis of memory and language disorders. Electrical stimulation over left temporoparietal cortical sites for select patients disrupted pattern discrimination, not naming; stimulation of the right brain elicited only anomia.

In case with early injury and critical lesions, language shifted to the noninjured right brain whole visuospatial functions received lower developmental priority and were delegated, totally or in part to the damaged left brain. Early left brain injury [LTE] may promote interhemispheric transfer of functions and honor the developmental priority of language, whereas spatial functions may be assigned to the damaged brain.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02920-01SB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Ischemia: ET-1 and Nitric Oxide

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

P.I.:I.	M. Spatz, M.D.	Section Chief	SB, NINDS
	R. M. McCarron, Ph.D.	Microbiologist	SB, NINDS
Others:	Yoshihide Yasuma, M.D.	Visiting Fellow	SB, NINDS

COOPERATING UNITS (if any)

Alois Strasser, DVM, University of Veterinary Medicine, Vienna, Austria

LAB/BRANCH

Stroke Branch

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	0.15	PROFESSIONAL:	0.15	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have explored the possibility of imbalance between the production of endothelin-1 (ET-1) and nitric oxide (NO) which may exacerbate brain injury. ET-1 a known potent vasoconstrictor peptide and NO, an endothelial relaxing factor, are produced among other vasoactive mediators in the vascular endothelium and in other cells in the brain. It has been suggested that an imbalance between the production of ET-1 and NO in the endothelium may play a role in cerebrovascular disorders. In particular, the observed increased ET-1 levels in plasma and/or cerebrospinal fluid (CSF) of patients with stroke, hypertension and vasospasm implicate ET-1 in the pathogenesis of cerebrovascular disease process. The aim of this study was to establish whether cerebral ischemia leads to CSF elevation of ET-1 which could be additionally altered by inhibition of NO synthase (NOS). Bilateral carotid artery occlusion (15 min) alone or with release (5-120 min) in gerbils served as a model for cerebral ischemia. The treatment consisted of either nitro-L-arginine (NLA) or D-nitro-L-arginine methyl ester (D-NAME) 40 mg/kg b.w.) in Ringer's solution (0.5ml) or the solvent alone given intraperitoneally 4 hr prior to the induction of ischemia. Similarly treated sham-operated animals were used as controls. Systemic blood pressure (SBP), cerebral blood flow (CBF), temporal and rectal temperature were continuously monitored in anesthetized (halothane 1.5% and NO₂/O₂ 1%) and spontaneously ventilated gerbils. CSF was obtained from the cisterna magna at the end of each experimental period. The level of ET-1 in CSF was measured by radioimmunoassay. Temperature was not significantly affected by either treatment. SBP was only elevated in NLA-treated animals during pre- and postischemia (NLA 77.67 ± 1.88 and 85.33 ± 1.75 mm Hg, respectively; others 66.67 ± 3.45 and 72.5 ± 2.17 mm Hg, respectively), although ischemia induced a similar rise in SBP in all groups of gerbils. CBF was reduced (0.5% of control) during ischemia in all groups. NLA treatment but not D-NAME prevented the initial complete recovery of CBF during reperfusion (7.5 min). In the CSF, the ET-1 level was raised 2-3 fold over controls in early reperfusion after 15 min of ischemia. The NLA-treated animals already showed the increased content of ET-1 in the CSF after ischemia alone with reperfusion. This is the first demonstration of changes in ET-1 content of CSF in ischemia/reperfusion. NOS inhibition produces an early and persistent rise in ET-1 levels of CSF in addition to SBP elevation and incomplete recovery of CBF during ischemia/reperfusion.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02912-02SB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Observations on the Brain-Derived Neurotrophic Factor (BDNF) in the Cerebral Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Igor Klatzo, M.D. Senior Scientist SB, NINDS

COOPERATING UNITS (if any)

Stanley Wiegand, Ph.D., Susan Croll, Ph.D., Regeneron Pharmaceuticals, Inc, Tarreytown, NY

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS: 0.1	PROFESSIONAL: 0.05	OTHER: 0.05
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Our studies have generally been focused on the nature of defensive and regenerative mechanisms which can be activated in response to ischemic injury, as well as on potential therapeutic manipulation of such defensive responses. In these studies we were able to demonstrate participation of the BDNF in the development of enhanced neuronal resistance to ischemic injury, demonstrable 3 days after induction of the spreading depression to the cortex. In therapeutic trials, BDNF was administered via the microcanule inserted stereotactically into the ventral thalamic region of the rat subjected to cardiac arrest cerebral ischemia (CAI). The delivery of the BDNF commenced 3 days before the CAI and lasted until the sacrifice of the animal at 7 days after cardiac arrest. Using specific monoclonal antibody for GABA demonstrated a striking proliferation and sprouting of GABA-ergic terminals in the ventral thalamic nucleus in the vicinity of the site of BDNF delivery. Also, there was a conspicuous preservation of GABA-ergic neurons of the nucleus reticularis thalami on the side of BDNF application.

The project was completed as of January 3, 1995.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02885-03 SB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Regulation of Gene Activity in Astrocytes		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
P.I.	Michael Brenner, Ph.D.	Special Expert
Others:	Qi Zhang, Ph.D.	Visiting Fellow
	John Hallenbeck, M.D.	Chief
		SB, NINDS
		SB, NINDS
		SB, NINDS
COOPERATING UNITS <small>(if any)</small> X.Liu, D-L Yao, LENP; A Messing, Sch Vet Med, U Wis, Madison, WI; J Schwartz,CNB; S-J Kim, CPCP, NCI; E. Radany, Dep Rad. Oncol., U. Mich., Ann Arbor, MI; J. Segovia, Dep. Neurosci, Mexico City, A. Zimmer, I CR NIMH		
LAB/BRANCH Stroke Branch		
SECTION Clinical Investigation Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	2.03	PROFESSIONAL: 1.53
		OTHER: 0.50
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>Current studies of the central nervous system (CNS) are assigning an increasing number of activities to <u>astrocytes</u>, many of which are potentially relevant to stroke. However, nearly all of these suggested functions are based on observed correlations, and many of these were made on cultured cells, whose properties may differ from those <i>in vivo</i>. As an alternative approach to understanding astrocyte function, we are studying <u>transcriptional regulation</u> of the human gene encoding glial fibrillary acidic protein (GFAP), the major component of astrocyte intermediate filaments. By studying GFAP transcription, insights may be gained into mechanisms governing development, reaction to injury, and cell specificity. A second goal is to use identified astrocyte-specific transcription elements to direct expression of other genes in astrocytes. This enables testing of the roles of specific factors in CNS function; and may produce disease models. Transcriptional studies have focused primarily on identifying factors that act at a consensus <u>AP-1 site</u> that is essential for GFAP transcription. Since proteins encoded by the <u>jun</u> and <u>fos</u> proto-oncogene families are known to modulate transcription via AP-1 sites, their presence in a GFAP expressing astrocytic cell line was examined. Analyses included <u>gel mobility shift assays</u>, "shift Westerns" and detection of the specific mRNAs by <u>Northern</u> analysis. Preliminary results show a correlation between GFAP transcription and the presence of c-Jun, JunD, and Fra-2, but not with JunB, c-Fos, FosB or Fra-1. Working with a human astrocytoma cell line, we have found a strong correlation with GFAP gene activity and the presence of <u>JunD</u> and <u>Fra2</u>. This result raises the possibility that the cell specificity of GFAP expression is due in part to a requirement for this particular combination of AP-1 components. However, cotransfection of GFAP reporter genes along with various Jun and Fos expression vectors has failed to demonstrate such a requirement. Applications of our GFAP gene analyses to studies of brain function have been carried out in collaborations with other laboratories. These projects include effects on stroke and development of overproduction of the inflammation-related cytokines TGF-beta 1 and TNF-alpha; investigation of the role of astrocytes in brain function by analyzing the effects of their timed ablation mediated by the herpes simplex virus thymidine kinase or <i>E. coli</i> cytosine deaminase transgenes; production of mouse glioma models through expression of oncogenes; gene therapy for Parkinson's disease via expression of tyrosine hydroxylase; a feasibility study for constructing a regulatable transgene; and studies of the functional role of GFAP itself through its overexpression and <u>gene knock-out</u>.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02886-03 SB									
PERIOD COVERED October 1, 1994 through September 30, 1995											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Quantification of Neurologic Deficit Progression in Acute Stroke Patients											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I:</td> <td style="width: 33%;">T. DeGraba, M.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">SB, NINDS</td> </tr> <tr> <td>Others:</td> <td>J. Hallenbeck, M.D.</td> <td>Chief</td> <td>SB, NINDS</td> </tr> </table>			P.I:	T. DeGraba, M.D.	Senior Staff Fellow	SB, NINDS	Others:	J. Hallenbeck, M.D.	Chief	SB, NINDS	
P.I:	T. DeGraba, M.D.	Senior Staff Fellow	SB, NINDS								
Others:	J. Hallenbeck, M.D.	Chief	SB, NINDS								
COOPERATING UNITS (if any) B. Kelly, M.D. and A. Dutka, M.D., Dept. of Neurology, National Naval Medical Center, Bethesda, MD											
LAB/BRANCH Stroke Branch											
SECTION Clinical Investigation Section											
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892											
TOTAL STAFF-YEARS: 1.04	PROFESSIONAL: 0.71	OTHER: 0.33									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input checked="" type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input checked="" type="checkbox"/> (a2) Interviews		
<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input checked="" type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Stroke has been traditionally regarded as a catastrophic event in which maximal damage to brain tissue occurs almost immediately. Recently, clinical and animal research has revealed that the ultimate degree of <u>tissue damage</u> in a stroke is not determined in the first few minutes but instead evolves over a period of hours to days. Amplification of excitotoxic neurotransmitter release, progressive intracellular calcium accumulation, blood-brain barrier compromise, and regional inflammation all may play a role in delayed neuronal death. In conjunction with monitoring physiologic variables, including blood pressure and oxygen saturation, careful observation of clinical neurologic progression may provide an understanding of the "window of opportunity" for acute interventional therapy. In meeting the primary objective of this study 75 consecutive stroke patients were admitted to the National Naval Medical Center (NNMC) within 24 hr of the onset of <u>cerebral ischemic symptoms</u>. A record was kept of the progression of <u>clinical deficits</u> over the first 48 hr. A standardized examination (the <u>NIH Stroke Scale</u>) was performed on admission and every 8 hr for 48 hr. The patients were monitored in the neurology ICU with a continuous recording of blood pressure, heart rate, and oxygen saturation obtained over this same period. The goal of analysis was to identify the percent of NNMC patients who would develop significant progression in the acute postischemic period. Subgroup analysis showed that patients with initial NIH stroke scores of ≥ 10 were more likely to show progression ($P = 0.001$) than those with scores of < 10. Additionally, the presence of atrial fibrillation and a higher minimal arterial blood pressure were also associated with a higher risk of progression ($P = 0.007$ & $P = 0.042$ respectively.)</p>											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02887-03 SB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Activation of Cytokines, Leukocytes, and Endothelium After Acute Cerebral Ischemia		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
P.I:	T. DeGraba, M.D.	Senior Staff Fellow SB, NINDS
Others:	J. Hallenbeck, M.D.	Chief SB, NINDS
	R. McCarron, Ph.D.	Research Microbiologist SB, NINDS
	M. Spatz, M.D.	Section Chief SB, NINDS
	L. Penix, M.D.	Fellow SB, NINDS
COOPERATING UNITS <small>(if any)</small> B. Kelly, M.D., Dept. of Neurology NNMC, V. Aletich, M.D., Dept. of Radiology, M. Foust, M.D., N. Bakalar, M.D., T. Porter, M.D., Dept. of Psychiatry, NNMC		
LAB/BRANCH Stroke Branch		
SECTION Clinical Investigations Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: <div style="display: flex; align-items: center;"> <div style="flex: 1;">1.16</div> <div style="flex: 1;"> PROFESSIONAL: 0.83 </div> <div style="flex: 1;"> OTHER: 0.33 </div> </div>		
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>Effective management of <u>acute stroke patients</u> has remained elusive to the present date. Recent evidence indicates that increased <u>cytokine</u> levels and <u>leukocyte and endothelial cell activation</u> may play a major role in secondary neuronal injury after acute focal cerebral ischemia. The purpose of this investigation is to more clearly characterize the role of the inflammatory response after ischemic injury in humans with regard to its causal influence on secondary neuronal injury and predictive value for long-term functional outcome. Blood samples are drawn from acute stroke patients on admission and serially for the first 7 days. Serial neurologic exams are being performed and MRI scan of the head is done within the first 3 days of admission to determine infarct size. Depression scales are done within the first 14 days. All patients are being seen at 90 days after the ischemic event for follow up at which time blood cytokine levels, neurologic outcome scales, and depression scales are being performed. Through analysis of the advent and duration of cytokine activation, we hope to establish a correlative relationship between the postischemic inflammatory response and neuronal injury. Given the published work demonstrating neuronal protection after ischemia in animal models with antagonists of leukocyte activation and of the inflammatory pathways, we expect these results to establish a temporal window for future drug trials in reducing infarct size after acute stroke. In addition, since clinical outcome in stroke is also dependent on rehabilitation effort, the incidence of <u>depression</u> in stroke patients becomes an important variable in long-term outcome. Thus, we will observe the incidence of depression in stroke patients as it relates to the volume and location of cerebral infarction. A novel approach of correlating <u>sleep architecture</u> in stroke patients with the incidence of mood disturbance will be performed by obtaining a <u>poly-somnogram</u> in patients 3-6 months after the ischemic event. A comparison will be made between patients with and without depression. Polysomnograms will also be compared against those of patients with primary depression (who display a very characteristic sleep pattern). It is hypothesized that the mood disturbance in stroke patients may actually be a result of altered sleep patterns caused by the neuronal injury. This may lead to a new understanding of the etiology of mood disorders in stroke patients and aid in their treatment.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02888-03 SB																				
PERIOD COVERED October 1, 1994 through September 30, 1995																						
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Cytokine, Leukocyte and Endothelium Activation in Risk Factors for Stroke																						
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">P.I:</td> <td style="width: 40%;">T. DeGraba, M.D.</td> <td style="width: 30%;">Senior Staff</td> <td style="width: 15%;">SB, NINDS</td> </tr> <tr> <td>Others:</td> <td>J. Hallenbeck, M.D.</td> <td>Chief</td> <td>SB, NINDS</td> </tr> <tr> <td></td> <td>R. McCarron, Ph.D.</td> <td>Research Microbiologist</td> <td>SB, NINDS</td> </tr> <tr> <td></td> <td>M. Spatz, M.D.</td> <td>Section Chief</td> <td>SB, NINDS</td> </tr> <tr> <td></td> <td>L. Penix, M.D.</td> <td>Fellow</td> <td>SB, NINDS</td> </tr> </table>			P.I:	T. DeGraba, M.D.	Senior Staff	SB, NINDS	Others:	J. Hallenbeck, M.D.	Chief	SB, NINDS		R. McCarron, Ph.D.	Research Microbiologist	SB, NINDS		M. Spatz, M.D.	Section Chief	SB, NINDS		L. Penix, M.D.	Fellow	SB, NINDS
P.I:	T. DeGraba, M.D.	Senior Staff	SB, NINDS																			
Others:	J. Hallenbeck, M.D.	Chief	SB, NINDS																			
	R. McCarron, Ph.D.	Research Microbiologist	SB, NINDS																			
	M. Spatz, M.D.	Section Chief	SB, NINDS																			
	L. Penix, M.D.	Fellow	SB, NINDS																			
COOPERATING UNITS <small>(if any)</small> B. Kelly, M.D., A. Dutka, M.D., Dept. of Neurology, NNMC, V. Aletich, M.D., Dept of Radiology NNMC; C. Cunningham, M.D., Dept. of Vascular Surgery, R. Hargraves, M.D., Dept. of Neurosurgery, NNMC																						
LAB/BRANCH Stroke Branch																						
SECTION Clinical Investigation Section																						
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892																						
TOTAL STAFF-YEARS: 1.26	PROFESSIONAL: 0.92	OTHER: 0.34																				
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input checked="" type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input checked="" type="checkbox"/> (a2) Interviews													
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<input type="checkbox"/> (a1) Minors																						
<input checked="" type="checkbox"/> (a2) Interviews																						
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p> The major <u>risk factors</u> which are associated with an increased incidence of <u>stroke</u> have been known for many years. However, the basic mechanisms by which these factors lead to the increased risk are not fully understood. Preliminary studies indicate that <u>activation of the immune system</u> by risk factors for stroke (hypertension, hypercholesterolemia, diabetes and age) increases the risk of endothelial activation and the formation of intravascular thrombosis. By measuring the levels of cytokine and monocyte, macrophage and endothelial cell activation in the stroke-prone population and age matched controls without risk factors, an attempt will be made to characterize those factors which potentially increase the risk for activation of brain vessel endothelium as well as preparing the brain tissue (including the cerebral vasculature) for a hyperactive inflammatory response to an ischemic insult. In addition, although disease is a major cause of stroke in the U.S., no radiographic findings related to the stenosis nor specific morphologic features of the atherosclerotic plaque have been useful in predicting which will become symptomatic and which will remain asymptomatic. In this study, we are analyzing carotid endarterectomy surgical specimens from symptomatic and asymptomatic patients for leukocyte adhesion molecules on the plaque endothelial cells using immunofluorescence staining. Blood drawn at the time of pre-operative testing is being examined for leukocyte and endothelial cell activation by <u>fluorescence activated cell sorting</u> (FACS) and baseline cytokine levels. It is hypothesized that the local release of cytokines and the expression of endothelial cell surface leukocyte receptors play a major role in the conversion of an asymptomatic plaque to a symptomatic one. Understanding the role of <u>cytokines</u>, leukocyte activation, and endothelial interaction in promoting the cerebral ischemic state may lead to a novel approach in future stroke prevention regimens. </p>																						

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02889-03 SB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Spreading Depression in Cardiac Arrest Cerebral Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	I. Klatzo M.D.	Senior Scientist	SB, NINDS
Others	C.A. Ruetzler	Biologist	SB, NINDS

COOPERATING UNITS (if any)

L.P.Penix, M.D., Epilepsy Research Branch, NINDS

LAB/BRANCH

Stroke Branch

SECTION

Cerebrovascular Pathophysiology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	0.1	PROFESSIONAL:	0.05	OTHER:	0.05
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The role of the spreading depression (SD) was investigated in rats subjected to cardiac arrest cerebral ischemia (CAI). The SD was induced by application of KCl either on the exposed dura of the parietal cerebral cortex or by KCl perfusion through the hippocampus. Three days later, the animals underwent the CAI. With regard to the hippocampus unilateral perfusion with KCl regularly resulted in induction of the SD in the ipsilateral hippocampus, associated with marked elevation of glutamate. No such effect was observed in rats in which KCl had been substituted with physiologic saline solution. Animals with hippocampal KCl perfusion, followed 3 days later by CAI, showed significant protection of CA1 pyramidal neurons on the side of the perfusion. No such effect was observed in Krebs-Ringer perfused rats. The protective effect of KCl on CA1 pyramidal neurons was evident also following the cortical application, although the effect was more bilateral. The SD induced 3 days before CAI resulted in a marked reduction in the susceptibility of rats to audiogenic seizures (AuSz) when tested 24 hr after cardiac arrest insult.

To elucidate the protective nature of the SD, the brain tissue was studied in rate at various time intervals following induction of the SD and in various relevant control conditions. Our studies indicated a striking stimulation of protein synthesis in the hemisphere ipsilateral to SD, which was demonstrable only in animals with SD induction 3 days earlier. Elevation of protein synthesis was absent in rats sacrificed 1 or 7 days after SD induction and in all control groups of rats.

The project was completed on January 3, 1995.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02856-04 SB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Hibernation - A New Approach to Stroke Therapy		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>		
P.I: J.M.Hallenbeck, M.D. Others: K. Tasaki, M.D. M. Brenner, Ph.D. R. McCarron, Ph.D. M. Spatz, M.D. T. Ohtsuki, M.D. D. Dawson, Ph.D. N. Gentile, M.D.	Chief Guest Researcher Special Expert Research Microbiologist Research Medical Officer Visiting Fellow Visiting Fellow Guest Researcher	SB, NINDS SB, NINDS SB, NINDS SB, NINDS SB, NINDS SB, NINDS SB, NINDS SB, NINDS
COOPERATING UNITS <small>(if any)</small> L. Sokoloff, M.D., C. Kennedy, M.D., G. Dienel, Ph.D., LCM; H. Gainer, Ph.D., H. Jaffe, Ph.D., LNC/NINDS		
LAB/BRANCH Stroke Branch		
SECTION Clinical Investigations Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892-4128		
TOTAL STAFF YEARS: 5.99	PROFESSIONAL: 4.74	OTHER: 1.25
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>Efforts to develop effective measures for the treatment of stroke have generally been based on the implicit assumption that one, or at the most several, factors control the progressive brain injury that occurs during the early hours of focal brain ischemia. Postischemic progression of brain damage appears to be extremely multifactorial. There is a finite probability that the assumption underlying most therapeutic stroke trials that seek to identify a dominant or controlling factor that determines post-ischemic progression of brain damage is incompatible with the fundamental nature of the problem. Postischemic progression of brain damage may be the result of a constellation of minor causes and the quest for a dominant or controlling cause would then be ultimately futile. Unconventional approaches may be required to arrest cellular destruction in brain ischemia.</p> <p>This project continues to investigate <u>mammalian hibernation</u>, a state of natural tolerance to severely reduced blood flow and oxygen delivery. Efforts to isolate and identify the factor or factors that regulate the <u>controlled metabolic depression and tolerance of profound brain ischemia</u> that forms the essence of natural hibernation are in progress. Such factors with <u>pleiotropic effects</u> may have benefit in the <u>treatment of progressive brain damage</u> in human stroke that is characterized by loss of homeostatic control due to activation of a multitude of pathophysiological postischemic events. The existence of regulatory factors in hibernation is supported by several findings that render passive submission to the effect of ambient temperature unlikely: (1) The onset and rate of development of bradycardia and reduced oxygen consumption during the transition to hibernation is rapid and precedes a more gradual drop in body temperature. (2) Regulation of enzyme function and gene expression that contributes to preservation of homeostasis during hibernation has been demonstrated. (3) Artificially induced <u>hypothermia</u> leads to rapid death in animals otherwise able to tolerate the same degree of hypothermia during natural hibernation. The identification of these putative control mechanisms may enable us to prevent or minimize the breakdown of homeostasis and cellular damage in cerebral ischemia in other species.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02865-04 SB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Interactions Between Cerebrovascular Endothelial Cells and Blood Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	R.M. McCarron, Ph.D.	Microbiologist SB, NINDS
Others:	J.M. Hallenbeck, M.D.	Chief SB, NINDS
	M. Spatz, M.D.	Section Head SB, NINDS
	Y. Yasuma, M.D.	Guest Researcher SB, NINDS
COOPERATING UNITS (if any) A-L. Siren, Department of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD		
LAB/BRANCH Stroke Branch		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF-YEARS:	2.15	PROFESSIONAL: 1.85 OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The continuing studies presented here examine the role of <u>monocytes</u> (MO) derived from both <u>hyper-tensive</u> and <u>normotensive</u> rats in interactions with cerebral <u>microvascular endothelial cells</u> (EC) and their potential role as critical components in vascular disorders such as stroke. Adhesion of resting MO from SHR and WKY rats to untreated cultures of syngeneic cerebral microvascular EC were not significantly different. Activation of MO by culture with LPS or IL-1 beta and TNF-alpha up-regulated Mac-1 expression on SHR MO to a greater degree than was observed with WKY MO. Treatment of SHR MO resulted in a greater up-regulation of adhesion to both syngeneic and allogeneic EC than was observed with WKY MO. The degree of up-regulated adhesion to SHR EC monolayers was greater than was observed for WKY EC. Binding of untreated and activated MO to untreated EC monolayers could be partially inhibited (40-55% and 65-75%, respectively) by antibodies against $\beta 2$ integrins and ICAM-1. No inhibition was observed with antibodies to VLA-4. The binding of both resting and activated MO to stimulated EC was less inhibited (20-39%) by antibodies to $\beta 2$ integrins and ICAM-1; MO adhesion to stimulated EC was also partially inhibited (10%) by antibody to VLA-4. No differences in the percent inhibition by antibody treatment were observed between SHR and WKY MO cultures.</p> <p>The experiments presented here examine the contribution of hypertension to pathogenic mechanisms involving vascular endothelium. The findings demonstrate that MO adhesion to EC utilized $\beta 2$-integrins/ICAM-1 as well as other molecules. The findings also indicate the significantly higher adhesiveness expressed by MO from hypertensive rats as compared to normotensive rats. These studies suggest that hypertension enhances responsiveness of MO to inflammatory factors that promote adhesion. Subsequent MO interaction with endothelium via cytokines may be important in the genesis of stroke and may contribute to pathological changes seen in stroke and reperfusion injury.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 N5 02832-05 SB
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunochemical Observations on Neurotransmitter Changes in Global Cerebral Ischemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: I. Klatzo, M.D. Senior Scientist SB, NINDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Stroke Branch		
SECTION Section of Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFFYEARS: 0.1	PROFESSIONAL: 0.05	OTHER: 0.05
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Immunohistochemical observations on <u>GABA</u> and <u>glutamate decarboxylase (GAD)</u> in rats subjected to <u>cardiac arrest cerebral ischemia (CACI)</u> revealed strikingly early changes in the <u>immunoreactivity</u> of GABAergic neuronal elements expressed in the widespread swelling and increased GABA and GAD immunostaining of GABAergic terminals and boutons. These changes appeared to be generally reversible with the exception of the nucleus reticularis thalami (NRT) which showed 80% neuronal loss. GABAergic terminals in the adjacent ventral thalamic nuclei (VTN) showed, approximately 7 days after their initial disintegration, a sprouting of new terminals, which reached its peak 1 month after ischemia. This coincided with the cessation of audiogenic seizures and the return to the normal paired-pulse stimulation patterns in the hippocampus, indicating a return of GABA_A inhibitory function. The hybridization assays with GAP-43 revealed strong mRNA expression limited to the NRT of rats sacrificed 7 days after CACI. The described correlations between morphologic evidence of sprouting of GABAergic terminals, and clinical cessation of susceptibility to audiogenic seizures, as well as electrophysiologic demonstration of the return of GABA_A inhibitory function in the hippocampus indicate the regenerative effort of the brain tissue subjected to ischemia and provide criteria for evaluating various therapeutic measures in future studies. </p> <p style="margin-top: 20px;"> The project was completed on January 3, 1995. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02776-07 SB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Production of Experimental Allergic Encephalomyelitis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: R.M. McCarron, Ph.D.

Microbiologist

SB, NINDS

Others: M. Spatz, M.D.

Section Chief

SB, NINDS

COOPERATING UNITS (if any)

Dr. M.K. Racke, NIB, NINDS

Dr. R.S. Fujanami, Dept. Neurol., Univ. of Utah, Salt-Lake City, UT

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was temporarily suspended for FY95.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02777-07 SB												
PERIOD COVERED October 1, 1994 through September 30, 1995														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Human Cerebromicrovascular Endothelium: Distinct Peptidergic Responses <i>in vitro</i>														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.:</td> <td style="width: 33%;">M. Spatz, M.D.</td> <td style="width: 33%;">Section Chief</td> <td style="width: 15%;">SB NINDS</td> </tr> <tr> <td>Others:</td> <td>R.M. McCarron, Ph.D.</td> <td>Microbiologist</td> <td>SB NINDS</td> </tr> <tr> <td></td> <td>N. Kawai, M.D.</td> <td>Visiting Fellow</td> <td>SB NINDS</td> </tr> </table>			P.I.:	M. Spatz, M.D.	Section Chief	SB NINDS	Others:	R.M. McCarron, Ph.D.	Microbiologist	SB NINDS		N. Kawai, M.D.	Visiting Fellow	SB NINDS
P.I.:	M. Spatz, M.D.	Section Chief	SB NINDS											
Others:	R.M. McCarron, Ph.D.	Microbiologist	SB NINDS											
	N. Kawai, M.D.	Visiting Fellow	SB NINDS											
COOPERATING UNITS (if any)														
LAB/BRANCH Stroke Branch														
SECTION Section of Neurocytobiology														
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892														
TOTAL STAFF YEARS: 0.7	PROFESSIONAL: 0.3	OTHER: 0.4												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input checked="" type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have previously established that <u>endothelin-1</u> (ET-1) induces receptor (ET_A) mediated cerebro-microvascular permeability changes and may function on a proinflammatory agent. Lately, we have also demonstrated that ET-1 and ET-3 modulates the <u>ion transport systems</u> (Na⁺-K⁺-ATPase and Na⁺/K⁺/Cl⁻-cotransport) in endothelial cells derived from capillaries of rat brain. The experiments described here examined the effect of endothelin (ET-1) on another ion transport pathway, namely sodium/hydrogen (Na⁺/H⁺ exchange system) in rat brain capillary endothelium (RBEC). </p> <p> ET-1, ET-2 and ET-3 stimulated Na⁺ uptake into REBC with similar half-maximal stimulation (EC₅₀) values (0.7, 0.6, and 1.1 nM, respectively). This reaction was inhibited by the Na⁺/H⁺ antiport inhibitor, N-(ethyl-N-isopropyl)-amiloride (EIPA). The selective endothelin A (ET_A) receptor-antagonist [Cyclo-D-Trp-D-Asp-Pro-D-Val-Leu BQ123], but not endothelin B (ET_B) receptor-antagonists[(Cys¹¹, Cys¹⁵)-ET-1 (IRL1038) or N-cis-2,6-dimethylpiperidinocarbonyl-L-γ MeLeu-D-trp (COOMe)-D-Nle-ONa (BQ788)], inhibited both ET-1 and ET-3 stimulated Na⁺ receptor mediation. The protein kinase C (PKC) activator [phorbol 12-myristate 13-acetate (MAO) failed to stimulate Na⁺ uptake. The calcium-calmodulin (CaM) inhibitor (W7) reduced ET-1 stimulate Na⁺ uptake by 50%, whereas the PKC inhibitor (staurosporine) had no effect, indicating that ET-1 stimulation of Na⁺/H⁺ antiport system is linked to a CaM-dependent and PKC-independent pathway. The present results further support the idea that endothelins play a role in regulating ion transport across the blood-brain barrier.. </p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02795-07 SB												
PERIOD COVERED October 1, 1994 through September 30, 1995														
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Human Cerebromicrovascular Endothelial Receptors														
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> <table style="width: 100%; border: none;"> <tr> <td style="width: 20%;">P.I.:</td> <td style="width: 30%;">M. Spatz, M.D.</td> <td style="width: 20%;">Section Chief</td> <td style="width: 30%;">SB NINDS</td> </tr> <tr> <td>Others:</td> <td>R.M. McCarron, Ph.D.</td> <td>Microbiologist</td> <td>SB NINDS</td> </tr> <tr> <td></td> <td>N. Kawai, M.D.</td> <td>Visiting Fellow</td> <td>SB NINDS</td> </tr> </table>			P.I.:	M. Spatz, M.D.	Section Chief	SB NINDS	Others:	R.M. McCarron, Ph.D.	Microbiologist	SB NINDS		N. Kawai, M.D.	Visiting Fellow	SB NINDS
P.I.:	M. Spatz, M.D.	Section Chief	SB NINDS											
Others:	R.M. McCarron, Ph.D.	Microbiologist	SB NINDS											
	N. Kawai, M.D.	Visiting Fellow	SB NINDS											
COOPERATING UNITS <small>(if any)</small>														
LAB/BRANCH Stroke Branch														
SECTION Section on Neurocytobiology														
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892														
TOTAL STAFF YEARS: 0.65	PROFESSIONAL: 0.25	OTHER: 0.4												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input checked="" type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK <small>(Use standard unrounded type. Do not exceed the space provided.)</small> <p>In view of the observed <u>ET-1</u> stimulation of <u>ion transport systems</u> in RBEC, it has been important to establish whether these systems also exist in microvascular endothelium derived from human brain (HBMEC). Uptake of $^{86}\text{Rb}^+$ ($0.2 \mu\text{C}/\text{well}$) as a tracer of K^+ uptake was determined in confluent HBMEC preincubated (30 min) with inhibitor or antagonist and incubated (5 min) alone or with ET-1 or ET-3 in serum-free medium at room temperature. The same procedures were used for HBMEC exposed to hypoxia (95% N_2, 5% CO_2) for 24 hr. ET-1 but not ET-3 dose-dependently increased K^+ uptake which was inhibited with BQ123 (ET_A receptor antagonist) but not with IRL1038 (ET_B receptor antagonist). Ouabain ($\text{Na}^+\text{K}^+\text{-ATPase}$ inhibitor) reduced the ET-1-stimulated K^+ uptake to a greater degree (94%) than bumetanide [$\text{Na}^+\text{K}^+\text{Cl}^-$ cotransport inhibitor] 30%. N-ethyl-n-isopropyl amelinide (EIPA) the inhibitor of N^+/H^+ exchange reduced the ET-1-stimulated K^+ uptake in the presence of bumetanide only. Verapamil, the inhibitor of Ca^{2+} channels decreased the ET-1 stimulated K^+ uptake in the presence of ouabain but not with bumetanide. In contrast, staurosporine [inhibitor of protein kinase c (PKC)] reduced the ET-1 stimulated K^+ uptake in the presence of bumetanide but not ouabain. Overnight exposure of HBMEC in nitrogen atmosphere dose-dependently augmented the ET-1 stimulation of K^+ uptake affecting the ouabain-sensitive K^+ uptake only.</p> <p>The data indicated that: 1) $\text{Na}^+\text{K}^+\text{-ATPase}$ activity and $\text{Na}^+\text{K}^+\text{Cl}^-$ cotransport are stimulated by ET-1 through activation of ET_A receptors in HBMEC; 2) the ET-1 stimulation of the $\text{Na}^+\text{K}^+\text{-ATPase}$ activity is mediated by Ca^{2+} ions and is linked to Na^+/H^+ exchange, whereas the $\text{Na}^+\text{K}^+\text{Cl}^-$ cotransport is linked to PKC; and 3) hypoxia amplifies the ET stimulation of $\text{Na}^+\text{K}^+\text{-ATPase}$ activity. This study represents the first demonstration of ionic transport systems in HBMEC. The observed ET-1 modulation of $\text{Na}^+\text{K}^+\text{-ATPase}$ activity and $\text{Na}^+\text{K}^+\text{Cl}^-$ cotransport indicate that ET-1 may play a role in regulating electrolytes transport across the blood-brain barrier (BBB). The hypoxic augmentation of ET-1 stimulated $\text{Na}^+\text{K}^+\text{-ATPase}$ activity strongly suggests that ET-1 (released from vascular, blood and/or brain cells) may participate in the disturbances of water electrolytes homeostasis under pathological conditions such as ischemia.</p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02797-07 SB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cultures of Human and Rat Cerebromicrovascular Endothelium: Mechanisms of Endothelin Effects		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	N. Kawai, M.D.	Visiting Fellow SB NINDS
Others:	M. Spatz, M.D.	Section Chief SB NINDS
	R.M. McCarron, Ph.D.	Microbiologist SB NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Stroke Branch		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINDS, NIH Bethesda, Maryland 20892		
TOTAL STAFF YEARS: <div style="text-align: right;">1.35</div>	PROFESSIONAL: <div style="text-align: right;">0.95</div>	OTHER: <div style="text-align: right;">0.4</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%; text-align: center;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> The specific <u>ion transport systems</u> localized in the <u>brain capillary endothelium</u> have been implicated in the regulation of water-electrolyte homeostasis in the brain. This report that hypoxia alters K⁺ transport systems in cultured rat brain capillary endothelial cells (RBEC). Uptake of ⁸⁶Rb⁺ (a tracer for K⁺, 2.5 μCi/ml) into RBEC was measured in HEPES-buffered Medium 199 at room temperature for 10 min. Ouabain-sensitive and bumetanide-sensitive K⁺ uptake was defined as Na⁺K⁺-ATPase and Na⁺K⁺ Cl⁻ cotransport activity, respectively. Hypoxia (95% N₂/5% CO₂, 24 hr) reduced (61% of control) the Na⁺K⁺-ATPase activity, whereas it increased (149% of control) the Na⁺K⁺-cotransport activity (respective control values = 4.6 and 5.5 nmol/mg protein/min, n=8). Oligomycin, a metabolic inhibitor (1 μg/ml), similarly affected both ion transport systems in a time-dependent manner, which caused an increased (133% of control) total K⁺ uptake. Oligomycin also increased the rate of K⁺ efflux up to 129% of the control without altering the total intracellular K⁺ content (values in μmol/mg protein; control = 96, oligomycin = 0.93). The oligomycin augmented Na⁺K⁺Cl⁻ cotransport activity was reduced by the protein tyrosine kinase inhibitors (genistin, 50 μM; herbimycin A, 10 μM) without being affected by an inhibitor of protein kinase C (bisindolylmaleimide, 500 nM) or protein kinase A (H8, 20 μM), indicating the involvement of protein-tyrosine phosphorylation. The data indicate that the up-regulated K⁺ uptake during hypoxia is due to an increased Na⁺K⁺Cl⁻ cotransport activity. It is suggested that a similar mechanism may play a role in the disturbance of water-electrolyte homeostasis described in ischemic brain. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02689-11 SB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Endothelin and Prostanoid Production in Cerebromicrovascular Endothelium

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	M. Spatz, M.D.	Section Chief	SB NINDS
Others:	R.M. McCarron, Ph.D.	Microbiologist	SB NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was suspended for FY95 due to the very limited availability of human brain endothelial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02623-12 SB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cerebral Ischemia and Edema: Biogenic Amines, Nitric Oxide, Peptides		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	M. Spatz, M.D.	Section Chief SB NINDS
Others:	R.M. McCarron, Ph.D.	Microbiologist SB NINDS
	Y. Yasuma, M.D.	Visiting Fellow SB NINDS
	N. Kawai, M.D.	Visiting Fellow SB NINDS
COOPERATING UNITS (if any) D. Stanimirovic, M.D., Ph.D., National Research Council of Canada, Ottawa, Canada; Alois Strasser, DVM, University of Veterinary Medicine, Vienna, Austria		
LAB/BRANCH Stroke Branch		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.9	PROFESSIONAL: 0.5	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Recently, we demonstrated that the involvement of <u>nitric oxide</u> (NO) in the delayed initial recovery of <u>cerebral blood flow</u> (CBF) after <u>transient cerebral ischemia</u> is associated with altered dopamine (DA) metabolism. In this study, the effect of <u>L-arginine</u>, the precursor of nitric oxide, on ischemic dopamine release from the striatum was investigated in Mongolian gerbils subjected to bilateral carotid artery occlusion (15 min) alone or with reflow (2hr). Dopamine and its metabolites were measured in the striatal extracellular space dialysate after continuous perfusion (2µl/min) of artificial extracellular fluid in the presence or absence of 15 mmol/liter L- or D-arginine or 1mmol/liter nitro-L-arginine. L-arginine but not D-arginine increased the striatal content of dopamine in pre- and postischemia whereas it lowered the levels of dopamine and 3-methoxytyramine induced by ischemia. In contrast, nitro-L-arginine reduced the preischemic levels of dopamine and 3,4-dihydroxyphenylacetic acid, and had no effect on the ischemic release of dopamine. These findings indicate that L-arginine stereospecifically modified the ischemic release and metabolism of dopamine. The data also suggest that the basal level of nitric oxide is not involved in dopamine release during ischemia but may participate in regulating dopamine release under physiological conditions.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02357-17 SB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cerebral Ischemia: Neurotransmitters, Metabolism, and Therapy		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; padding: 5px 0;"> P.I.: M. Spatz, M.D. Section Chief SB NINDS </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH Stroke Branch		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Our previous studies suggested that the relative resistance of young gerbils may be attributed to neuronal function. On the other hand, the <u>ischemic induction of heat shock protein</u> HSP72 has been suggested to play a protective role against neuronal injury. To elucidate further, the mechanisms responsible for the observed are dependent upon susceptibility to brain ischemia, we investigated the effect of ischemia on HSP72 expression at both transcriptional and translational levels in the hippocampus of young and adult gerbils.</p> <p>The HSP72 RNA expression was observed in all hippocampal areas within 3 hr of reflow, reaching a maximum by 8 hr of reflow in both young and adult gerbils. However, a much stronger <i>in situ</i> hybridization was observed at 1 hr of reperfusion in the hippocampus of young than that seen in adult animals. A progressive decrease in HSP72 mRNA expression was seen in various areas of the hippocampus except CA1. At 48 hr, the persisting expression of mRNA in CA1 was more marked in young than adult gerbils. The appearance of HSP72 protein was detected at a later time than that observed for mRNA. The most striking difference was seen in CA1 neurons which showed a more marked accumulation of HSP72 protein in young than that observed in adult animals.</p> <p>These studies strongly suggest that the immature neurons possess an endogenous tolerance to ischemia that may be related to higher transcriptional activity.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02324-19 SB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Blood-Brain Barrier: *In Vitro* Model for the Study of Cerebrovascular Endothelial Permeability

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

P.I.: R.M. McCarron, Ph.D.

Microbiologist

SB, NINDS

Others: M. Spatz, M.D.

Section Chief

SB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was temporarily suspended for FY95.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02922-01 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Apoptosis in Tumors of the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Katherine Wood, Ph.D.

Visiting Associate, SNB, NINDS

Others: Edward Oldfield, M.D.
Abha Saxena, Ph.D.Chief, SNB, NINDS
Visiting Associate, SNB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Molecular Biology Unit, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.25

PROFESSIONAL: 0.75

OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Solid tumors in the central nervous system represent the third largest cause of cancer related deaths in the 20 to 35 age group and primary intracranial tumors are the most type of solid tumor in children. The genotoxic agents used in conventional therapy can cause severe side effects and damage, particularly to the developing brain. We are analyzing the role of apoptosis in tumor cell killing to evaluate the potential use of apoptosis-associated genes in therapy for CNS tumors. We have analyzed medulloblastoma cell lines and determined that radiation sensitivity is a function of p53 expression levels and the p53-dependent induction of apoptosis. The introduction of p53 protein into tumor cells lacking functional p53 increased radiation sensitivity by initiating apoptosis and was toxic to these cells. We are currently investigating recurrent PNET for loss of functional p53, the transient delivery of p53 to tumors cells as a means of reducing the effective therapeutic dose of radiation and/or to initiate an effective therapy for recurrent tumors or those that refractive to conventional therapy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02859-04SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Programmed Cell Death in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard J. Youle, Ph.D.

Chief, Biochemistry Section, SNB, NINDS

Others: Katherine A. Wood, Ph.D.

Visiting Fellow, SNB, NINDS

Yi Te Hsu, Ph.D.

Special Volunteer, SNB, NINDS

Joann Castelli

Pre-IRTA

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have studied programmed cell death in the nervous system and the biochemical mechanism of apoptosis in general. To approach the nervous system, more sensitive and *in situ* methods are needed to identify cells undergoing programmed cell death. We have developed two new methods to identify apoptotic cells under the microscope. (1) We have found that thymocyte-programmed cell death can be followed morphologically with Nomarski optics and that the thymocyte death resembles neuronal cell death. The morphologic analysis of nuclear disintegration has allowed us to test whether cell death is due to production of a toxic factor or due to the loss of a protective factor. Using the new microscopic method to identify apoptosis, the nuclei in the heterokaryons were found to follow the original and distinct fate of the parent cells and not to transfer apoptosis nor viability between nuclei. This new method also allowed us to identify apoptosis as the method of cerebellar granule cell death after MPP⁺ treatment *in vitro*. (2) We have also developed a molecular detection method to measure DNA strand breaks *in situ*. This allows us to examine brains of animals undergoing neurodegenerative changes during ischemia, MPTP treatment, and during development. This new method has allowed us to determine the role apoptosis plays during development and during various disease states of the nervous system. (3) We have developed monoclonal antibodies against apoptosis regulatory genes Bcl-2, Bcl-x and Bax to probe the regulation of apoptosis *in vitro* and *in vivo*.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02855-04 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interstitial Therapy with Targeted Protein Toxins for Malignant Brain Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Douglas Laske, M.D.	Senior Staff Fellow, SNB, NINDS
Others:	Eward H. Oldfield, M.D.	Chief, SNB, NINDS
	Richard J. Youle, Ph.D.	Chief, Biochemistry Section, SNB, NINDS
	Orhan Ilercil, M.D.	Clinical Associate, SNB, NINDS
	David Katz, M.D.	Neuropathologist, OD, NINDS
	Nicholas Patrons, M.D.	Radiologist, CC

COOPERATING UNITS (if any)

Department of Radiology, CC

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	0.2	PROFESSIONAL:	0.2	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are investigating a new approach for the treatment of brain tumors which utilizes new delivery approach for distribution of a class of potent, targeted anti-cancer compounds, called targeted protein toxins. Preclinical *in vitro* and *in vivo* experiments of toxins targeted to the transferrin receptor and epidermal growth factor (EGF) have demonstrated significant antitumor activity against a variety of tumor types including malignant gliomas. New methods of drug delivery have been developed to deliver these agents to brain tumors, and *in vivo* imaging method are being developed to demonstrate drug distribution in patients. We have initiated a dose escalation trial of regional therapy with the targeted protein toxin transferrin-CRM107 (Tf-CRM107) for the treatment of recurrent malignant brain tumors. Tf-CRM107 is a conjugate of human transferrin (Tf) and diphtheria toxin with a point mutation (CRM107). Tf-CRM107 binds to the transferrin receptor, which facilitates iron uptake and is present in higher number on tumor cells than on the normal cells of the brain, and the diphtheria toxin mutant kills these tumor cells to which the Tf-CRM107 binds. The purpose of the study is to evaluate the toxicity of Tf-CRM107 when delivered by intratumoral and peritumoral slow interstitial infusion in a dose escalation schedule and to assess antitumor activity in these patients. Twenty-seven patients with malignant brain tumors refractory to standard therapy (surgery, radiation \pm chemotherapy) have been treated. Results indicate that therapy with Tf-CRM107 effects tumor responses, without severe neurologic or systemic toxicity. A multicenter Phase II study is being planned. In addition, an EGF-target toxin is being prepared for clinical trial. Synergism between targeted protein toxins and other antitumor reagents including standard chemotherapy drugs and retinoids is under investigation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02854 - 04 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Establishing the Physiology of Syringomyelia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	John D. Heiss, M.D.	Senior Staff Fellow, SNB
	Nick Patronas, M.D.	CC, Radiology
	Thomas Shawker, M.D.	CC Radiology
	William Kammerer, M.D.	CC Anesthesiology
	Alec Eidsath, Ph.D.	RR, BEIP

COOPERATING UNITS (if any)

Diagnostic Radiology Department, CC
Anesthesiology Department, CC, BEIP

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	1.03	PROFESSIONAL:	0.93	OTHER:	0.10
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to establish the mechanism(s) of progression of communicating syringomyelia. Communicating syringomyelia usually accompanies abnormalities at the craniocervical junction. Measurement of intraventricular pressure, intrathecal pressure, and intrasyrinx pressure is providing data which elucidate the hydrodynamic mechanism(s) of progression of syringomyelia. Radiographic testing, including MRI flow studies and ultrasonography, is demonstrating how pathologic anatomy alters normal cerebrospinal fluid flow. The effect of posterior fossa craniectomy, upper cervical laminectomy, and duraplasty on cerebrospinal fluid flow and pressure, syrinx size, and neurological function is being evaluated. Twelve patients have been treated. Only one patient had communication between the 4th ventricle and the syrinx. Despite obstruction of CSF pathways at the foramen magnum, phase and cine-MRI demonstrated pulsatile syrinx and cervical subarachnoid CSF flow. Ultrasonographic measurements demonstrated tonsillar descent, cord and syrinx constriction, and caudal syrinx fluid flow during systole. CSF pressure measurements showed that intracranial pulse pressure was transmitted well to the cervical subarachnoid space and syrinx but poorly to the lumbar thecal space. Because intracranial pressure is transmitted despite obstruction of the subarachnoid space at the foramen magnum, we conclude that the cerebellar tonsils and the brainstem act on a partially enclosed spinal subarachnoid space to generate cervical subarachnoid CSF pressure waves. These waves compress the spinal cord from without, not from within, as has previously been considered to occur, to propel the syrinx fluid downward with each heartbeat. Syrinx progression occurs as a consequence. Craniocervical decompression and duraplasty improved cerebrospinal fluid flow at the foramen magnum in all patients. All syringes decreased in size following surgery. All pressure measurements have been performed without complication, including postoperative measurement of cervical and lumbar pressure.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02850- 04 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Therapy of Disorders of the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.

Chief, SNB, NINDS

Others: Stuart Walbridge

Biologist, SNB, NINDS

R. Michael Blaese, M.D.

Chief, Cellular Immunology, MB, NCI

COOPERATING UNITS (if any)

National Cancer Institute, Bethesda, Maryland
Genetic Therapy, Gaithersburg, Maryland

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.05

PROFESSIONAL:

0.05

OTHER:

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

New approaches to transfer genetic material into tumor and normal central nervous system (CNS) tissue is being explored. The mechanisms involved in effecting antitumor activity using the suicide gene transfer approach are investigated. Normal and tumor vasculature, choroid plexus epithelium, and than normal CNS structures are being targeted. New viral vectors, including adenoviruses, are being evaluated for potential therapeutic approaches. A clinical trial for treating patients with recurrent malignant brain tumors with a retroviral vector containing the gene for Herpes simplex thymidine kinase (HsTk) and intravenous ganciclovir (GCV) was completed. The results indicate that 1) the producer-cell approach can be used successfully without toxicity in human brain tumors, 2) antitumor activity occurs in some patients, 3) limited gene transfer into tumor cells occurs with this approach for delivery and distribution, and 4) "bystander effects" probably underlie the antitumor activity. The results highlight the need for improved methods of drug delivery and distribution in solid tissues. They also suggest that with improvements in gene delivery and development of techniques to select patients with tumors that are more likely to respond, this approach may have clinical utility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02868-04 SNB															
PERIOD COVERED October 1, 1994 Through September 30, 1995																	
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Semi-Chronic Intracortical Electrical Stimulation of the Visual Cortex of a Blind Volunteer																	
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> <table style="width: 100%; border: none;"> <tr> <td style="width: 20%;">PI:</td> <td style="width: 40%;">Conrad Kufta, M.D.</td> <td style="width: 40%;">Medical Officer, SNB, NINDS</td> </tr> <tr> <td>Others:</td> <td>Daniel O'Rourke, M.D.</td> <td>University of Pittsburgh, PA</td> </tr> <tr> <td></td> <td>Martin Bak</td> <td>Electrical Engineer, LNLC, NINDS</td> </tr> <tr> <td></td> <td>Edward Schmidt, Ph.D.</td> <td>Biological Engineer, LNLC, NINDS</td> </tr> <tr> <td></td> <td>F. Terry Hambrecht, M.D.</td> <td>Head, Neuroprothesis, NINDS</td> </tr> </table>			PI:	Conrad Kufta, M.D.	Medical Officer, SNB, NINDS	Others:	Daniel O'Rourke, M.D.	University of Pittsburgh, PA		Martin Bak	Electrical Engineer, LNLC, NINDS		Edward Schmidt, Ph.D.	Biological Engineer, LNLC, NINDS		F. Terry Hambrecht, M.D.	Head, Neuroprothesis, NINDS
PI:	Conrad Kufta, M.D.	Medical Officer, SNB, NINDS															
Others:	Daniel O'Rourke, M.D.	University of Pittsburgh, PA															
	Martin Bak	Electrical Engineer, LNLC, NINDS															
	Edward Schmidt, Ph.D.	Biological Engineer, LNLC, NINDS															
	F. Terry Hambrecht, M.D.	Head, Neuroprothesis, NINDS															
COOPERATING UNITS <small>(if any)</small> University of Pittsburgh																	
LAB/BRANCH Surgical Neurology Branch, NINDS																	
SECTION Clinical Neurosurgery Section																	
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland																	
TOTAL STAFF YEARS: 0.18	PROFESSIONAL: 0.18	OTHER: 0															
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) Human subjects</td> <td><input checked="" type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews								
<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither															
<input type="checkbox"/> (a1) Minors																	
<input type="checkbox"/> (a2) Interviews																	
SUMMARY OF WORK <small>(Use standard unrounded type. Do not exceed the space provided.)</small> This project is designed to evaluate the feasibility of a visual <u>prosthesis</u> for totally blind individuals by stimulating chronically implanted <u>microelectrodes</u> in the visual cortex. A 42-year-old woman who has been <u>blind</u> for 22 years was implanted with an array of 38 electrodes in the visual cortex. Stimulation of individual electrodes produced sensation of light called phosphenes. Phosphenes were produced with 34 of the 38 electrodes with currents that were 100 to 1000 times lower than had been reported for surface stimulation of the <u>visual cortex</u> . Additional blind patients need to be tested before we will know if <u>intracortical microstimulation</u> (ICMS) of the visual cortex is a feasible technique for producing a visual prosthesis. However, all the tests performed to date indicate that ICMS may be feasible.																	

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02812-06 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pentobarbital Effects on Damage of the Primate Brain by Fractionated Whole Brain Radiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.

Chief, SNB, NINDS

Others: Calvin Hawkins

Bio Lab Technician, SNB, NINDS

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.13

PROFESSIONAL:

0.08

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Radiation therapy remains the single most effective treatment for malignant brain tumors, but in many cases, toxicity to normal brain impedes therapeutic doses sufficient for local control to be achieved. A substantial effort has been directed toward overcoming the unfavorable side effects of brain tumor radiation therapy. Small animal studies in our Branch indicate that pentobarbital anesthesia during cerebral irradiation reduces the toxicity of the ionizing radiation. Although mechanisms of this phenomenon remain unclear, it may arise from suppression of brain synaptic activity or metabolism. After baseline MRI scans of the brain and neuroendocrine testings, primates (*Macaca mulatta*) underwent whole brain X-irradiation in 10 daily fractions, 360 rads each (total dose of 3600 rads). The monkeys in the study group were anesthetized with pentobarbital during the irradiation, whereas the animals in the control group received ketamine. Each group consists of 6 animals. Neuroendocrine testing and MRI scan follow-up studies are performed at 3, 6 12, 18 and 24 months after irradiation to detect potential differences in the degree of brain injury in the two groups.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02813-06 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacokinetics of Direct Brain Infusion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	Douglas W. Laske, M.D.	Senior Staff Fellow, SNB, NINDS
	Oscar Sanchez, M.D.	Clinical Associate, SNB, NINDS
	Mark Corthesy, M.D.	Visiting Associate, SNB, NINDS
	John Pace, M.D.	Clinical Associate, SNB, NINDS
	Paul Morrison, Ph.D., Robert Dedrick, Ph.D.	Biomedical Engineering, RR

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	5.45	PROFESSIONAL:	3.42	OTHER:	2.03
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

For many compounds (neurotrophic factors, antibodies, growth factors, genetic vectors, enzymes) minimal diffusion in the brain severely limits drug distribution after direct drug administration in to brain parenchyma. We systemically investigated convection, molecular transport with bulk flow of fluid, to enhance the distribution of large and small molecules, indium¹¹¹-transferrin (In¹¹¹-Tf; MW 80,000) and C¹⁴-sucrose (MW 359), by maintaining a pressure gradient during interstitial infusion to generate bulk flow through the brain interstitium. The volume of distribution (V_d) containing $\geq 1\%$ of infusate concentration increased linearly with the infusion volume (V_i) for In¹¹¹-Tf ($V_d/V_i = 6.1$) and C¹⁴-sucrose ($V_d/V_i = 14.1$). 24 hr after infusion, the distribution of In¹¹¹-Tf increased, became more homogeneous, and penetration into gray matter occurred. By using convection to supplement simple diffusion, greatly enhanced distribution of large and small molecules can be achieved in the brain while achieving drug exposure orders of magnitude greater than systemic exposure. Convection-enhances distribution was shown to be an effective technique to homogeneously deliver large and small molecules in the gray matter of rats and non-human primates. the infusion of molecular selectively toxic to certain subsets of neurons is now being investigated as a potential new therapeutic strategy for Parkinson's disease and for seizures. Continuous perfusion of most of the cerebral hemisphere of monkeys was achieved for several days with an implanted controllable pump.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02814-06 SNB																		
PERIOD COVERED October 1, 1994 through September 30, 1995																				
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Genetic Abnormalities in Primary Glial Tumors																				
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Iqbal Ali, Ph.D.</td> <td style="width: 40%;">Special Expert, SNB, NINDS</td> </tr> <tr> <td>Others:</td> <td>Abha Saxena, Ph.D.</td> <td>Visiting Associate, SNB, NINDS</td> </tr> <tr> <td></td> <td>Joan Barrick, B.S.</td> <td>Biologist, SNB, NINDS</td> </tr> <tr> <td></td> <td>Edward H. Oldfield, M.D.</td> <td>Chief, SNB, NINDS</td> </tr> <tr> <td></td> <td>William Stettler-Stevensen, M.D.</td> <td>NCI</td> </tr> <tr> <td></td> <td>James Robertson, M.D.</td> <td>Chairman, Dept. of NS, University of Tennessee</td> </tr> </table>			PI:	Iqbal Ali, Ph.D.	Special Expert, SNB, NINDS	Others:	Abha Saxena, Ph.D.	Visiting Associate, SNB, NINDS		Joan Barrick, B.S.	Biologist, SNB, NINDS		Edward H. Oldfield, M.D.	Chief, SNB, NINDS		William Stettler-Stevensen, M.D.	NCI		James Robertson, M.D.	Chairman, Dept. of NS, University of Tennessee
PI:	Iqbal Ali, Ph.D.	Special Expert, SNB, NINDS																		
Others:	Abha Saxena, Ph.D.	Visiting Associate, SNB, NINDS																		
	Joan Barrick, B.S.	Biologist, SNB, NINDS																		
	Edward H. Oldfield, M.D.	Chief, SNB, NINDS																		
	William Stettler-Stevensen, M.D.	NCI																		
	James Robertson, M.D.	Chairman, Dept. of NS, University of Tennessee																		
COOPERATING UNITS <small>(if any)</small> University of Tennessee, Memphis, Tennessee LCMB, NCI, NIH, Bethesda, Maryland																				
LAB/BRANCH Surgical Neurology Branch, NINDS																				
SECTION Molecular Biology Unit, SNB, NINDS																				
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892																				
TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0																		
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"><input checked="" type="checkbox"/> (a) Human subjects</td> <td style="width: 33%;"><input checked="" type="checkbox"/> (b) Human tissues</td> <td style="width: 33%;"><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews											
<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither																		
<input type="checkbox"/> (a1) Minors																				
<input type="checkbox"/> (a2) Interviews																				
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>The work of the molecular biology unit has focused on the identification of <u>genetic alterations</u> that contribute to the initiation and progression of <u>malignant tumors</u> in the central nervous system. A multifaceted approach has been taken to help determine the genetic alterations responsible for the expression of a malignant phenotype in CNS tumors. Studies of the expression of growth factors and growth factor receptors in glial tumors showed elevated expression of basic fibroblast growth factor (BFGF) and the BFGF high affinity receptor and increased levels of epidermal growth factor and alpha-platelet derived growth factor. Matrix metalloproteins and metalloproteases (MMPs) are postulated to be involved in the regulation of tumor invasiveness. Cellular invasiveness can be modulated by altering the balance between activated MMPs and their inhibitors. A new project to investigate the role of type IV collagen and collagenases, located in the basement membrane, in determining invasive activity in pituitary tumors has been initiated. Analyses of genetic alterations demonstrated a loss of heterozygosity on chromosomes 10 and 17 in a significant percent of glial tumors. Although the p53 gene locus lies on chromosome 17, deletions in this gene were rare, implying that a second, as yet unidentified, tumor suppressor gene is located on the short arm of chromosome 17, distal to the p53 locus, and that inactivation of the second tumor suppressor gene may function in the initiation or progression of astrocytic neoplasms. In Nelson's tumors loss of heterozygosity of chromosome 17 and point mutation in p53 were identified in two of six tumors. Metastatic lesions from primary non CNS tumors and recurrent tumors exhibited alterations in p53 suggesting that loss of wild-type p53 contributes to tumor progression and spread. The cyclin-dependent kinase 4 inhibitor gene, p16, on chromosome 9, has been implicated in progression of malignancy in tumors. A study of deletion of the p16 gene was undertaken in DNA extracted from glioblastoma multiform (GBM) tissue. Homozygous deletion of p16 was common in primary GBM. Comparisons between primary and recurrent tumors show that a higher percentage of recurrent GBM show deletion on p16 which extend toward the telomere and the centromere.</p> <p>Project completed October 2, 1995.</p>																				

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02815-06 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Pituitary Corticotroph Adenomas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Iqbal Ali, Ph.D.	SNB, NINDS
Others:	Joan Barrick, B.S.	Biologist, SNB, NINDS
	Abha Saxena, B.S.	Biologist, SNB, NINDS
	Edward Oldfield, M.D.	Chief, SNB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Molecular Biology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	0	PROFESSIONAL:	0	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project was completed October, 1995.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02823-06 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibody-Toxin Conjugates for the Treatment of Human Brain Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard J. Youle, Ph.D. Chief, Biochemistry Section, SNB, NINDS

Others: Doug Laske, M.D. Senior Staff Fellow, SNB, NINDS
Edward H. Oldfield, M.D. Chief, SNB, NINDS
Cynthai Sung, Ph.D. Staff Fellow, PEIB

COOPERATING UNITS (if any)

Hafslund Nycomed, PEIB

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	1.33	PROFESSIONAL:	1.33	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have engineered immunotoxins into exquisitely cell type specific reagents with promise for cancer therapy. Exploring their applications *in vivo* we have found that 1) there are powerful pharmacologic barriers that limit protein access to tumor cells; 2) this problem is exacerbated in the brain where the blood-brain barrier prevents macromolecule movement into the brain tissue; 3) the plant and bacterial toxins used for construction of immunotoxins are highly immunogenic and soon after treatment antibodies arise that inactivate reagent. Thus, to overcome the problems of delivery and immunogenicity, we have pursued regional delivery of immunotoxins to the brain as away to treat brain tumors. Since cancer can spread and grow in the CSF, a condition known as leptomeningeal carcinomatosis, immunotoxins were initially injected directly into the cerebral spinal fluid to access tumor cells and were found to kill 99% to 99.9% of the tumor cells *in vivo* with occasional animals cured. An intriguing dose limiting toxicity was found specifically related to this route of administration. Purkinje cells were killed by diphtheria toxin derived immunotoxin guinea pigs and ricin derived immunotoxins in rats and monkeys. Another protein, called the eosinophil-derived neurotoxin is homologous to RNases A and also selectively kills Purkinje cells. 4) Comparing a family of homologous RNases we found 5000-fold variation in cytotoxicity. The molecular basis of toxicity was explored and cell binding, RNase inhibitor sensitivity and/or enzyme activity all appear to contribute. 7) We have determined the dose limiting toxicity of immunotoxins in three model species, guinea pigs, rats and rhesus monkeys and in man. 8) The pharmacology of a monoclonal antibody against the transferrin receptor, 454A12, coupled to recombinant ricin A chain was thoroughly studied in primates and man. Clearance from the CSF was biphasic and in humans, a somewhat larger clearance rate was found for the antitransferrin receptor immunotoxin than seen with other macromolecules possibly reflecting uptake by tumor cells. A potentially large therapeutic window exists for intrathecal immunotoxins for cancer therapy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02781-07 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tissue Implantation in Parkinsonian Models

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	Marc Corthesy, M.D.	SNB, NINDS
	Alex Cummins, M.S.	Biologist, SNB
	Yitong Fu, M.D., Ph.D.	Visiting Fellow, SNB, NINDS

COOPERATING UNITS (if any)

David Jacobowitz, Clinical Neuropharmacology, NIMH, Charles Gerfen, Neurophysiology, NIMH, Ivan Mefford, Neurochemistry, NIMH

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

CNS Transplantation Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	1.83	PROFESSIONAL:	0.73	OTHER:	1.1
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Brain grafting and pallidotomy are being used to treat patients suffering from Parkinson's disease who are refractory to medical therapy. Behavioral recovery following caudate cavitation in Parkinsonian monkeys focused our attention on potentially beneficial host responses to grafting. Following injury, the CNS produces neurotrophic factors which promote neurite outgrowth and glial proliferation. We have explored the therapeutic potential of growth factors to preventing or reverse biochemical and behavioral deficits in rodent and primate models of Parkinson's disease. To deliver growth factors to gray matter we are developing methods for convection-enhanced direct infusion into the striatum. We used convection to enhance the distribution of large molecules injected into the striatum in rats, measured using immunohistochemistry and quantitative autoradiography. We are now exploring the continuously delivery of proteins such as BDNF, to the striatum of MPTP-lesioned monkeys. Recent electrophysiologic and anatomic studies have shown hyperactivity of neurons in the subthalamic and globus pallidum interna nuclei produce the symptoms of Parkinson's disease. Accordingly, we are exploring the use of excitatory amino acids to destroy the neurons of the globus pallidus interna in monkeys as a novel therapy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02739-09 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Laboratory Investigation of Central Nervous System Vascular Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Edward H. Oldfield, M.D.,	Chief, SNB, NINDS
Others:	Robert Boock, Ph.D.	Staff Fellow, SNB, NINDS
	Ryszard Pluta, M.D.	Visiting Associate, SNB, NINDS
	Tom Manski, M.D.	National Naval Medical Ctr
	John Heiss, M.D.	Senior Staff Fellow, SNB, NINDS

COOPERATING UNITS (if any)

Diagnostic Radiology Department, CC, Experimental Therapeutics Branch, NINDS; Surgery Branch, National Cancer Institute; National Naval Medical Center, Bethesda, Maryland

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.73

PROFESSIONAL:

2.63

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Endothelial-derived relaxation factor nitric oxide (NO) was shown to mediate autoregulation and chemoregulation of cerebral blood flow in primates and NO synthase (NOS) immunoreactivity was demonstrated in the nerve plexus in the adventitia of the circle of Willis in primates. In a primate model of subarachnoid hemorrhage (SAH) adventitial NOS disappeared on day 7 after SAH, concurrently vasospasm, suggesting that NO loss plays a role in the pathogenesis of cerebral vasospasm after SAH. Thus, direct replacement of NO should reverse the vasospastic effect of any NO loss. In the primate model of vasospasm, intra-arterial infusions of NO solution and NO donor solution reversed arteriographic cerebral vasospasm, significantly increased cerebral blood flow, and decreased cerebral blood flow velocity. These findings further support a central role of NO in the pathogenesis of cerebral vasospasm and suggest the potential of a regional NO therapy for cerebral vasospasm. We also have explored the effects of the putative agents of vasospasm, oxyhemoglobin and its breakdown product methemoglobin in cell culture. These examine the possibility that vasospastic agents, such as, endothelin, may be released from tissues exposed to oxyhemoglobin and methemoglobin. While exposure to hemoglobin does not directly increase endothelin levels, hypoxia, a condition associated with a decrease in cerebral blood flow, causes dramatic increases in endothelin. Thus, endothelin may be responsible for secondary ischemia-producing effects associated with vasospasm after SAH. The most common type of cranial dural arteriovenous fistulas was shown to be treated effectively by simple interruption of the intrathecal venous drainage, a much simpler and safer procedure than the prior management of these patients. The lasting efficacy of a simple surgical procedure for patients with spinal dural arteriovenous fistulas, interruption of the vein draining the fistula intradurally, was demonstrated. A new type of tumor associated with von Hippel-Lindau (VHL) syndrome, low grade adenocarcinoma of the endolymphatic sac, was identified and shown to occur in at least 19% of VHL patients.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02708-10 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vascular Permeability Factor/Vascular Endothelial Growth Factor in the CNS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Marsha Merrill, Ph.D., Biologist	SNB, NINDS	
Others:	John Heiss, M.D., Senior Staff Fellow	SNB, NINDS	Edward H. Oldfield, M.D., Chief
	Efstathios Papavassiliou, M.D., Vis. Fell.	SNB, NINDS	SNB, NINDS
	Nancy Edwards, B.A., Biologist	SNB, NINDS	
	Mima Bacic, M.D., Visiting Associate	SNB, NINDS	
	Abha Saxena, Ph.D., Visiting Associate	SNB, NINDS	
	Stuart Walbridge, Biologist	SNB, NINDS	

COOPERATING UNITS (if any)

Laboratory of Cardiovascular Science, NIA, NIH; National Naval Medical Center, Bethesda, MD

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Tumor Biology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.28

PROFESSIONAL:

3.48

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is a protein secreted by many cell types which has been shown in model systems unrelated to the nervous system to perform two major functions: it is an angiogenic, endothelial-specific growth factor, and it is a potent inducer of capillary permeability. We have proposed that VEGF/VPF is also a mediator of endothelial cell proliferation and capillary permeability in the normal and diseased central nervous system. Previous results from our laboratory have demonstrated that increased VEGF/VPF expression may underlie the increased permeability associated with brain tumor vessels and the resulting edema observed in that clinical setting. Hyperpermeability associated with brain tumors and the resulting brain edema is a significant cause of morbidity and mortality in patients with brain tumors. Our continuing goals are to define the function of VEGF/VPF in the central nervous system, to understand the extent to which altered expression of VEGF/VPF contributes to the symptomatology of certain pathologic conditions, to determine the factors responsible for regulation of VEGF/VPF expression, and to develop strategies for modifying the activity of VEGF/VPF in appropriate clinical settings. The major approaches which we are using are: 1) the use of cell cultures to determine what factors regulate VEGF/VPF expression; 2) a nonreplicating adenovirus containing the VEGF/VPF gene which allows us to examine the effects of overexpression of VEGF/VPF in otherwise normal rodent brain; 3) two models of brain injury in rodents (stab and freeze lesion); and 4) analysis of tumor vessel permeability using quantitative autoradiography in a rodent brain tumor model to assess the efficacy and mechanism of action of clinical treatments for this disease. Our findings from this year are: 1) When injected into the normal rodent brain, an adenovirus coding for the VEGF gene greatly increases the permeability of capillaries in the region and disrupts the blood-brain barrier. This effect is specific for VEGF/VPF, as viruses coding for other proteins do not have this effect. 2) In two models of brain injury (stab and freeze lesions), VEGF/VPF expression increased dramatically and specifically in reactive astrocytes associated with the lesions, reaching peak expression between three and seven days after injury and persisting for at least three weeks. These observations indicate that astrocytes react to injury by increasing VEGF/VPF expression, and that VEGF/VPF may be an important factor in wound healing in the CNS. 3) VEGF/VPF is up-regulated in normal astrocytes and in brain tumor cells by serum growth factors and by hypoxia. 4) the steroid dexamethasone alters VEGF/VPF activity at two levels, inhibiting both the growth factor-dependent induction of VEGF/VPF expression and the ability of VEGF/VPF to increase capillary permeability. Our experiments have shown that both of these effects occur through the glucocorticoid receptor, a finding that has important implications for the proper management of brain tumor patients.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02674-11 SNB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Engineering Cell-Type Specific Toxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard J. Youle, Ph.D.	Chief, Biochemistry Section, SNB, NINDS
Others:	You-Neng Wu, Ph.D.	Visiting Associate, SNB, NINDS
	Ester Boix, Ph.D.	Visiting Fellow, SNB, NINDS
	Veena Vasandani, Ph.D.	Visiting Fellow, SNB, NINDS
	Shalindra Saxena, Ph.D.	Visiting Associate, SNB, NINDS

COOPERATING UNITS (if any)

Alfacell, Bloomfield, New Jersey

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	2.7	PROFESSIONAL:	2.1	OTHER:	0.6
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monoclonal antibodies selectively bind tumor cell differentiation antigens *in vitro* and *in vivo*. Since natural effector mechanisms often do not mediate killing of monoclonal antibody bound cells we have devised methods of linking extremely toxic proteins to the antibodies to selectively kill tumor cells. We have succeeded in developing several new approaches to apply immunotoxins in vivo. (1) Cloning toxins, then altering their structure at the gene level to decrease non target cell toxicity; (2) preparation of genetically engineered immunotoxins for clinical trials of human brain tumor patients; (3) prevention of an immune response against immunotoxin with anti-CD4 antibodies; (4) use of human cytotoxic proteins such as RNase linked to antibodies to selectively target cells; and (5) understanding the mechanism of human RNase neurotoxins; 6) Discovering that RNases block HIV infection *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02454-15 SNB
PERIOD COVERED October 1, 1994 through September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Human Pituitary Tumors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Edward Oldfield, M.D. Chief, SNB, NINDS		
COOPERATING UNITS (if any) Developmental Endocrinology Branch, NINDS Diagnostic Radiology, CC		
LAB/BRANCH Surgical Neurology Branch, NINDS		
SECTION Clinical Neurosurgery Section, CNP		
INSTITUTE AND LOCATION NINDS, National Institutes of Health, NINDS		
TOTAL STAFF YEARS: 1.2	PROFESSIONAL: 1.0	OTHER: 0.20
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We continue to investigate new approaches to diagnose <u>Cushing syndrome</u> (CS), make the diagnosis of CS, and localize small <u>pituitary adenomas</u> to aid in the diagnosis and treatment of patients with CS. The results indicate that bilateral simultaneous inferior petrosal sinus (IPS) sampling. Distinguishes patients with ectopic ACTH secretion from those with pituitary adenomas with nearly 100% accuracy. Repeat transsphenoidal surgery is successful in eliminating the <u>hypercortisolism</u> of Cushing's disease in about 70% of patients. This therapy for patients with Cushing's disease after previous pituitary surgery previously had not been examined. Repeated sella exploration in the early postoperative period in patients who did not respond to the first operation was shown to be successful in most patients who received it. The subset of patients who are most likely to have success with early repeat surgery can be selected based on the findings during the first operation. MRI scanning with and without gadolinium-EDTA was used to evaluate patients with Cushing's disease preoperatively. This technique permitted identification of the adenoma in only 55% of patients with surgically-proven microadenomas. Pituitary adenomas were detected in 10# of 100 normal subjects with MRI scanning with contrast. Intraoperative ultrasound, using a prototype 12 Mhz prob developed for transsphenoidal surgery, was shown to detect and localizing very small tumors in the pituitary gland during surgery. The use of these techniques resulted in curative pituitary surgery in 100% of a large group of children with Cushing's disease. Hyponatremia, which frequently occurs in the early postoperative period after pituitary surgery was shown to be produced by abnormal regulation of vasopressin secretion and abnormal excessive thirst.		





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